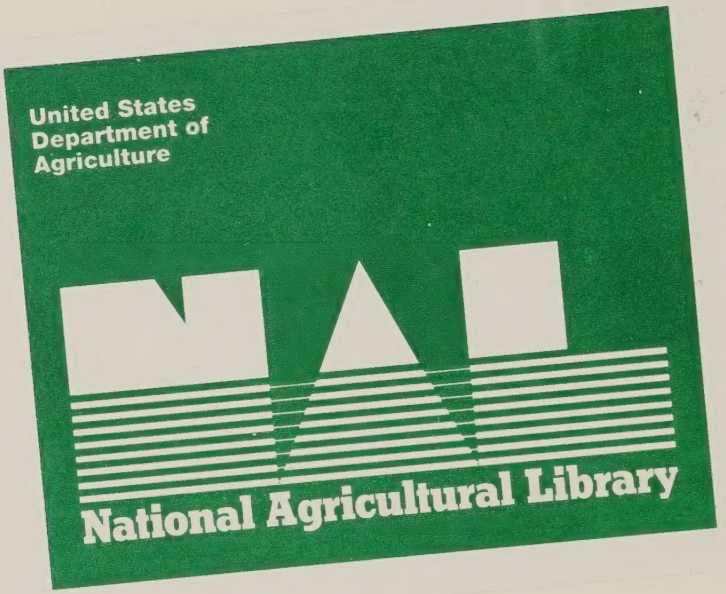
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Agriculture
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U. S. DEPARTMENT OF AGRICULTURE

STATUS OF INSECT VIRUS RESEARCH IN ARS

JAMES E. WRIGHT

NATIONAL PROGRAM STAFF

United States Department of Agriculture
Agricultural Research Service
Mid South Area

Mid South Area
Boll Weevil Physiology Research
P. O. Box 5367
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January 31, 1985

SUBJECT: Responses to Questionnaire Regarding Status of Insect Virus Research in ARS

TO: Respondents

This compilation of responses gives an indication of the present research currently on-going in ARS. Some individuals may have been inadvertently not included and they are encouraged to submit their information to the NPS and also to the list included.

Careful perusal of the responses will reveal that ARS does not have a closely coordinated nor a dedicated group active in virus research area. However, the utilization of viruses remains a viable component for integration into pest management.

Pertinent research objectives:

- manipulation of phenotypic expression of virulence
- classical and genetic engineering of viral genomes for pest-management purposes
- fundamental research on use of viruses as vectors of insect genes

Your responses are appreciated and further dialog with NPS involving your research is encouraged.

JAMES E. WRIGHT, RL
Boll Weevil Physiology Research

Location and Scientists Background Information

Arizona, Phoenix
- Western Cotton Research Laboratory
  - Bell, M. R.

California, Fresno
- Stored Product Insects Research Laboratory
  - Kellen, W. R.
  - Vail, P.

Georgia, Tifton
- Insect Biology and Population Management Research Laboratory
  - Hamm, J. J.

Iowa, Ankeny
- Corn Insects Research Laboratory
  - Lewis, L. C.

Kansas, Manhatten
- U.S. Grain Marketing Research Laboratory
  - Johnson, D. E.
  - McGaughey, W. H.

Maryland, Beltsville
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  - Adams, J. R.
  - Dougherty, E. M.
  - Lynn, D. E.
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  - Shapiro, M.

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USDA, ARS
Western Cotton Research Laboratory
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Phoenix, AZ 85040 PH: FTS: 261-3524

2. Location:

Phoenix, AZ

3. Number and title of CRIS work unit:

5510-20230-004

4. Approach Element and Problem Definitions:

Crop Protection - Insect
Development of ecologically acceptable methods of crop protection from insect damage.

5. Estimated SY's:

Cotton Insect Pathology/Microbial Control: 1 SY
Insect Virus Research: ca. .2-.3 SY

6. Objectives of research:

Develop new and innovative methods of controlling cotton insects through the development of microbials that provide effective control. This includes methods of application, formulations to increase effectiveness, and integration of microbial control with other methods of control. Studies are also conducted toward determining basic pathological relationships of cotton insects and their pathogens.

7. Research priorities in your program:

Development of methods of control of cotton insect pests through the use of microbial insecticides. Recent priorities have been directed towards increasing the effectiveness of Bacillus thuringiensis (B. t.) and baculoviruses in the control of Heliothis spp. in cotton. The research includes the development and use of feeding stimulant baits, mixtures of B. t. and baculovirus for control, integration of B. t., baculovirus, and chemical insecticides, investigation of new methods of application, effects of formulation on microbial deposition and persistance on cotton plants.

8. Progress of current research in solving problems:

A feasable control method, utilizing a commercial bait formulation (COAX® - developed by this program) with a mixture of B. t. and virus was proven in several field tests, and accepted by industry and consumers. Recent chemical insecticide use has decreased the market for this method, but it will be available when needed.

Laboratory studies in use of mixtures of B. t. and baculovirus illustrate the degree of antagonism between these microbials and possible field effects.

Effects of dosage of B. t. on Heliothis growth rates and subsequent effects on susceptibility to chemical insecticides are being studied.

9. Significant research accomplishments in the past 3 years:

Field tests demonstrated efficacious control of Heliothis virescens in cotton, utilizing microbials applied by standard methods.

In-depth study of the effect of various doses of B. t. - baculovirus mixtures on growth and mortality of Heliothis virescens.

Development of the bait, COAX®, into a dust for use in certain situations.

10. Impact of research accomplishments on science and the general public:

The impact on science has been in the advancement of knowledge in the effects of microbials on Heliothis spp., plus creating an awareness of microbials as to their possible effects on other research programs. Also, the work spurred increased research by other scientists in the area of microbial control. The impact on general public has included the development of a commercial product, still on the market, and the demonstration of the use of microbial insecticides in insect control programs. The product, and its use, advanced awareness of microbials, created jobs, and increased the use of a safe insect control method.

11. Obstacles to achieving objectives:

Few scientists aware of field research methods and knowledge of microbials and what to expect from those pathogens under study. Further, new fast-acting pathogens would aid in developing new, more effective control methods. Research should include baculoviruses as well as other groups of microbials.

12. Future lines of needed research and plan for implementation:

I feel that the future line of needed research should include genetic engineering towards development of more potent pathogens - including representatives of several pathogen groups. However, we also need more information in field usage of present pathogens and the continued ability to evaluate new pathogens as they become available.

I don't know that ARS should be directly involved in the creation of "new" pathogens as much as a check on their feasability for use in control systems.

13. Research facilities and personnel needs:

Presently have facilities for applied research in evaluating pathogens in cotton insect control. To get into a team approach would require a great amount of increase in facilities, funding, equipment and personnel.

14. Extent of cooperation--names of persons and institutions:

Primary cooperation has been from industry.

Sandoz Corp.

Proctor and Gamble (Trader's Protein Division)

15. Titles of publications for the last 3 years:

Microbial Agents - Chap. 6 in Ag. Handbook No. 589. "Cotton Insect Management" 1982.

"Dispersal of Baculoviruses in the Environment"
Book chapter in Viral Insecticides for Biological Control - Edited by K. Maranorosch. In Press.

"Incorporating Varying Doses of Bacillus thuringiensis and a Heliothis baculovirus in diet: Effect on H. virescens and H. zea." 1983, Abstract, Soc. Invert. Pathology XVII Annual Mtg.

Microbial Control of Heliothis spp. in Cotton: Dosage and Management Trials. J. Geo. Ent. Soc. Accepted 3/84.

Cotton Leaf Perforator: Effect of Time Microbial Insecticides on Field Populations. JEE 75(6) 1982 1140-42.

1. **Scientist's name, address, and telephone number:**

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FTS - 467-5310

2. **Location:**

Fresno, CA

3. **Number and title of CRIS work unit:** 5302-20620-012

Development of Insect Pathogens for Control of Postharvest Pests of Dried Fruit and Nuts

4. **Approach Element and Problem Definitions:**

Approach Element 4.3.1. Develop improved methods for controlling losses caused by insect pests.

5. **Estimated SY's:**

1SY: (a) NRP 20620 : 100% (b) Approach Element 4.3.1. : 100 %

6. **Objectives of research:**

1. Develop new generation of pesticides that are nontoxic and persistent.
2. Develop new methods for controlling postharvest losses of agricultural products (Project area: 4.3.1.5, pathogens) utilizing microbials.

7. Research priorities in your program:

Annual postharvest losses of food is estimated at $31 billion. Means are needed for reducing or eliminating postharvest losses caused by insects.

1. The following is a list of the most important pests selected for research priority: Codling moth, Indianmeal moth, navel orangeworm, almond moth, Trogoderma sp., dried fruit beetles (Nitidulidae).

2. Insect viruses have been given priority since they appear to offer the best possibility for applied use on a commercial basis.

8. Progress of current research in solving problems:

Emphasis of current research has been to isolate, identify, and determine host relationships of several previously unreported isometric viruses isolated from Amyelois transitella in California. Data on virulence and cytopathology have been obtained. Studies indicate the viruses strongly influence larval mortality and adult longevity and fecundity. Viruses will be tested as possible introductions to experimental populations for long-term effect. Distribution of isometric viruses in natural populations is unknown. Baculoviruses have not been reported from this host. Current research also involves (1) study of the autodissemination of a granulosis virus baited in the pheromone of the Indianmeal moth (2) Efficacy of baculoviruses on dried fruit against the raisin moth, and (3) pathogens of the Medfly.

9. Significant research accomplishments in the past 3 years:

Determined the ultrastructure, pathology, and physicochemical characteristics of two small, isometric virus of Amyelois transitella. Quantitative data showed that sublethal doses of virus strongly influence the longevity and fecundity of adult A. transitella with chronic disease. Determined the thermoinactivation points and thermal resistance of larvae to calicivirus infection. Described the comparative pathogenicity of two new baculoviruses of Cadra figulilella. Host specificity and transmission were studied in laboratory tests.

10. Impact of research accomplishments on science and the general public:

Except for the study of honey bee diseases, the small isometric viruses of insects are relatively unknown. Yet, it is evident from our recent studies that isometric viruses are probably widely represented in pest species. The detection, identification, distribution and abundance of the viruses have not been previously studied. Their potential for microbial control of postharvest pests is under investigation, utilizing dusts baited with pheromone, as augmentations to enhance endemic disease with debilitative effects on population growth. Similar studies with a granulosis virus of the Indianmeal moth will provide new scientific data that are needed to evaluate the efficacy of baculoviruses for practical application and commercial development. Accomplishments will lead to a biorational technology that will provide safe, effective postharvest insect control without toxic residues and harmless to man and nontarget species.

11. Obstacles to achieving objectives:

(1) Development of suitable pathogens for postharvest pest control. (2) Mass rearing techniques for ecomonical virus production (3) Cost of obtaining acceptable safety data for nontarget species (4) Need to develop in vivo systems. (5) Need to encourage generic approval by EPA of all baculoviruses as safe for use by man and nontarget species.

12. Future lines of needed research and plan for implementation:

(1) Conduct studies on the detection, purification and identification of pathogenic insect viruses and evaluate efficacy for control of postharvest pests. (2) Investigate biology of in vitro systems. (3) Investigate virus genetics and possibility of engineering new, more virulent strains with desired host-range characteristics. (4) Need to develop data on chemical characterizatio (protein and nucleic acid analysis) of viruses of postharvest pests for accurate identification and detection. Some of these lines of investigation are already in progress at this laboratory, or have been initiated with cooperative agreements by Dr. Pat Vail.

13. Research facilities and personnel needs:

Plans have been developed to construct new research facilities. Current facilities are adequate for present staffing, but does not provide for future needs and growth. New facility is proposed for completion in the next 3-5 years.

14. Extent of cooperation--names of persons and institutions:

Dr. James Vaughn, USDA, Maryland; Dr. T. Jack Morris, U.C. (Berkley) Dr. Max Summers, Texas A&M (College Station); Dr. Carlo Ignoffo, USDA, Missouri; Dr. Ron Goodwin, USDA, Bozeman, Montana.

15. Titles of publications for the last 3 years:

(attached)

Kellen, W. R. and Hoffmann, D. F. 1981. A pathogenic nonoccluded virus in hemocytes of the navel orangeworm, Ameylois transitella (Pyralidae: Lepidoptera). J. Invertebr. Pathol 38, 52-66.

Kellen, W. R. and Hoffmann, D. F. 1981. Wolbachia sp. (Rickettsiales: Rickettsiaceae) a symbiont of the almond moth, Ephestia cautella: Ultrastructure and influence on host fertility. J. Invertebr. Pathol. 37, 273-283.

Hillman, B., Morris, T. J., Kellen, W. R., and Hoffmann, D. F. 1982. An invertebrate calici-like virus evidence for partial virion disintegration in host excreta. J. Gen. Virol. 60, 115-123.

Kellen, W. R. and Hoffmann, D. F. 1982. Dose-mortality and stunted growth responses of larvae of the navel orangeworm, Amyelois transitella, infected by chronic stunt virus. Environ. Entomol. 11, 214-222, 1982.

Kellen, W. R. and Hoffmann, D. F. 1983. Thermoinactivation of a calicivirus of the navel orangeworm and effect of high temperature on larval resistance. Environ. Entomol. 12, 605-609. 1983.

Kellen, W. R. and Hoffmann, D. F. 1983. Longevity and fecundity of adult Amyelois transitella (Lepidoptera:Pyralidae) infected by two small RNA viruses. Environ. Entomol. 12, 1542-1546.

Kellen, W. R. and Hoffmann, D. F. 1984. Occurrence of two baculoviruses in Cadra figulilella (Lepidoptera:Pyralidae). J. Invertebr. Pathol. 43, 439-440.

1. Scientist's name, address, and telephone number:

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2. Location:

Fresno, California

3. Number and title of CRIS work unit:

5302-20620-009-00D - Develop insect pathogens for infield and postharvest control of horticultural crop insect pests

4. Approach Element and Problem Definitions:

Objective 4:	Agricultural products/domestic marketing and export
Approach 3:	Losses-pests, spoilage, damage
Element 01:	Control losses-insect pests
Problem 1:	Insects cause an average 10 percent loss of harvested commodities and agricultural products, and such a loss cannot be tolerated.
Subproblem d:	We have not found ways to expand the use of biological insect control agents for practical control of loss-causing insects
Subproblem 1:	Codling moths reduce exportability of products to Japan, but we do not have effective quarantine treatments to control them.

5. Estimated SY's:

1.0

6. Objectives of research:

(1) To develop microbial agents for control of insects of postharvest significance in the field.

(2) To develop microbial control agents for the control of insects in storage, processing and marketing channels.

7. Research priorities in your program:

(1) To develop the codling moth granulosis virus as a control agent for this insect in walnuts and other commodities prior to harvest.

(2) To develop the Indianmeal moth granulosis (IMM-GV) as a control agent in dried fruits and nuts with emphasis on marketing channels.

(3) To develop other insect pathogens for the control of oriental fruit moth, almond moth, raisin moth, navel orangeworm and other insects of postharvest significance in fresh and dried fruits and nuts.

(4) To find economical production methods and formulations that afford protection (protectants) for extended periods in marketing channels.

(5) To satisfy registration requirements (i.e. safety and environmental testing) by developing necessary data through contractural or other type agreements.

8. Progress of current research in solving problems:

(1) An easy to produce and economical formulation of the IMM-GV has been been developed.

(2) The temperature stability of the formulation at storage temperatures over one year has been determined.

(3) The efficacy of the above formulation for IMM control on inshell and shelled almonds and raisins has been determined.

(4) The IMM-GV has been applied in a commercial situation and its efficacy and persistence in storage determined.

(5) Efficacy and persistence of the CM-GV on walnuts has been determined.

(6) B.t. has been tested for control of IMM and other insects on dried fruits and nuts.

9. Significant research accomplishments in the past 3 years:

(1) Three years of small scale testing with the codling moth GV have been completed as well as the quantative persistence in the field.

(2) Small and large scale tests of the IMM-GV formulation on various dried commodities have been completed.

(3) A simple and economical method for the production and formulation of the IMM-GV was developed.

10. Impact of research accomplishments on science and the general public:

(1) Chemical insecticide sprays for CM control often cause upsets in walnut aphid populations because of their undesirable side effects on a parasitoid which normally provided excellent control. Commercial development of the codling moth GV could provide a high degree of control, eliminate the need for chemical control, and allow the aphid-parasitorial complex to become stabilized at levels below the economic threshold.

(2) The development of a simple, economical and efficacious IMM-GV formulation could provide control of IMM on dried fruits and nuts for extended periods of time in the marketing channels. Postharvest handling and storage provides an excellent environment for pathogens as UV is not a factor and rarely does temperature become one. At the present time these commodities are not protected once they leave the processor and enter marketing channels. Over 90% of the infestations reported are solely due to IMM. Use of this virus would significantly reduce infestation levels in domestic and foreign marketing channels and also reduce the returns of commodities by wholesalers and consumers. Fumigant use could be significantly decreased.

11. Obstacles to achieving objectives:

(1) Lack of funds - this research presently is being supported with ARS at a level of $110,000 net.

(2) Need for safety tests for both the IMM-GV and CM-GV.

(3) A philosophical and practical commitment of ARS in recognizing the uniqueness of the postharvest situation as an excellent opportunity to use pathogens.

(4) In-vitro methods for safety tests and production of GV's are lacking - a breakthrough in the area by ARS would be a significant contribution to all aspects of insect virology and microbial control.

12. Future lines of needed research and plan for implementation:

(1) Due to the present status of fumigants the development of pathogens for control of postharvest insect control needs to be emphasized further. One microbial, Bacillus thuringiensis, has already been registered for use on grain. In the future we plan to:

(a) Isolate, propagate, characterize and determine the efficacy of other entomogenous viruses.

(b) Facilitate the development of data necessary for registration.

(c) Develop in-vitro replication systems for characterization, safety testing and propagating of viruses.

(d) Determine methods for integration of pathogens with other control strategies. Candidate pathogens would be integrated with other control strategies such as controlled atmospheres, hormones and their analogs, physical treatments and other biotic agents.

13. Research facilities and personnel needs:

(1) At present, laminar flow benches are housed in separate building from the pathology unit. These benches should be incorporated into the main building. Cost approximately $25,000.

(2) A high resolution photoscope having bright field, dark field, phase contrast and UV optics is needed for histological and histochemical investigations. Cost approximately $45,000.

14. Extent of cooperations - names of persons and institutions:

Dr. William R. Kellen, Horticultural Crops Research Laboratory
Dr. Max D. Summers, Texas A&M University
Walnut Marketing Board of California
Almond Advisory Board
Raisin Advisory Board
Sun Diamond Corporation
Tenneco West Corporation

15. Titles of publications for the last 3 years:

Cowan, D. K., P. V. Vail, M. L. Kok-Yokomi and F. E. Shreiber. 198_. A formulation of a granulosis virus of Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae): Efficacy, persistance, and influence on oviposition and larval survival. In press.

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2. **Location:**

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3. **Number and title of CRIS work unit:**

CRIS 7702-20240-010
Southern Corn Insects: Pathology

4. **Approach Element and Problem Definitions:**

2.4.05.1.M

5. **Estimated SY's:**

1

6. **Objectives of research:**

1) Establish the identity and incidence of pathogens infecting lepidopterous larvae and other agricultural pests.

2) Evaluate pathogens as potential biological control agents.

3) Field test procedures for integrating pathogens into insect management programs.

4) Investigate effects of pathogens on non-target and beneficial organisms.

5) Study the pathology of diseases caused by pathogens in host insects.

6) Monitor the occurrence of and develop methods for control of pathogens of laboratory colonies of insects used for other research programs.

7. Research priorities in your program:

1) Evaluation of viruses of Spodoptera and Heliothis and investigation of factors affecting host range and virulence of viruses in an effort to find more effective pathogens of fall armyworm and to find or develop a virus that is highly virulent to both fall armyworm and corn earworm.

2) Development of methods for integrating viruses into insect management programs such as application of viruses to crops through irrigation systems.

3) Cooperation with other scientists to obtain basic information on viruses of Spodoptera and Heliothis that is needed to improve these viruses through genetic engineering.

4) Evaluation of effects of pathogens on non-target organisms, especially beneficial insects such as parasites and predators.

8. Progress of current research in solving problems:

Determined that an isolate of Heliothis armigera nuclear polyhedrosis virus virus (NPV) from the USSR and another isolate from China will infect both Heliothis and Spodoptera but is not nearly as virulent for fall armyworm as for corn earworm. On the other hand, an NPV from Autographa californica will infect both species but does not develop normally in corn earworm.

A new type of virus isolated from fall armyworm would not infect corn earworm although a similar virus isolated from corn earworm would infect fall armyworm.

Demonstrated that the Heliothis NPV was more effective against corn earworm on a field corn hybrid with a tight husk extension than on a hybrid with a loose husk that was open at the tip.

9. Significant research accomplishments in the past 3 years:

Demonstrated that fall armyworm NPV was about equally pathogenic to fall armyworm and beet armyworm but it failed to produce infective polyhedra in beet armyworm. Plaque-purified variants from one isolate of fall armyworm NPV lost pathogenicity to beet armyworm while remaining pathogenic to fall armyworm. This study demonstrated biological differences associated with previously described genomic differences.

Demonstrated that the new type of virus isolated from fall armyworm can be transmitted by parasites but that it interferes with parasite development in infected hosts.

Demonstrated that pathogens of insect pests can be applied to corn through the irrigation water.

10. Impact of research accomplishments on science and the general public:

The demonstration of biological differences associated with genomic differences in viral isolates provides a tool for further research on factors affecting virulence and host range of insect viruses.

The ability to apply viruses and other entomopathogens through the irrigation system will reduce the cost of application and may make the treatment of field corn economically feasible.

11. Obstacles to achieving objectives:

12. Future lines of needed research and plan for implementation:

Determine relationship between population density of pest, virus concentration, and number of applications needed to suppress the increase in corn earworm populations on corn and to limit the production of aflatoxin on corn.

Study factors affecting virulence and host range of insect viruses.

13. Research facilities and personnel needs:

We need to maintain colonies of additional species of Spodoptera and Heliothis such as S. eridania, S. orinithogalli, and H. virescens in order to screen isolates of viruses for differences in host range and virulence.

ARS should be more involved in foreign exploration for pathogens of insect pests. This would provide additional viral isolates to use in genetic engineering in an effort to produce viruses with desirable host ranges. One way to accomplish this would be to send an insect pathologist with entomologists that are looking for parasites and predators of pests.

14. Extent of cooperation--names of persons and institutions:

Eloise Styer - University of Georgia
Clinton Y. Kawanishi - Environmental Protection Agency
Lambert Loh - University of North Carolina
Max D. Summers - Texas A&M University
Donald A. Nordlund - USDA-ARS
John R. Young - USDA-ARS
Brian A. Federici - University of California

15. Titles of publications for the last 3 years:

Loh, Lambert C., Hamm, John J., and Huang, Eng-Shang. Spodoptera frugiperda nuclear polyhedrosis virus genome: Physical maps for restriction endonucleases BamHI and HindIII. J. Virology 38:922-931. 1981.

Hamm, J. J., and Hare, W. W. Application of entomopathogens in irrigation water for control of fall armyworms and corn earworms (Lepidoptera: Noctuidae) on corn. J. Econ. Entomol. 75:1074-1079.

Hamm, John J. Relative susceptibility of several noctuid species to a nuclear polyhedrosis virus from Heliothis armiger. J. Invertebr. Pathol. 39:255-256. 1982.

Hamm, John J. Extension of the host range for a granulosis virus from Heliothis armiger from South Africa. Environ. Entomol. 11:159-160. 1982.

Hamm, John J., and Lynch, R. E. Comparative susceptibility of the granulate cutworm, fall armyworm, and corn earworm to some entomopathogens. J. Georgia Entomol. Soc. 17:363-369. 1982.

Loh, Lambert C., Hamm, John J., Kawanishi, Clinton, and Huang, Eng-Shang. Analysis of the Spodoptera frugiperda nuclear polyhedrosis virus genome by restriction endonucleases and electron microscopy. J. Virology 44:747-751. 1982.

Walker, Susan, Kawanishi, C. Y., and Hamm, J. J. Cellular pathology of a granulosis virus infection. J. Ultrastructure Res. 80:163-177. 1982.

Hamm, John J., Nordlund, Donald A., and Mullinix, B. G., Jr. Interaction of the microsporidium Vairimorpha sp. with Microplitis croceipes (Cresson) and Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae), two parasitoids of Heliothis zea (Boddie) (Lepidoptera: Noctuidae). Environ. Entomol. 12:1547-1550. 1983.

Hamm, John J. Invertebrate pathology and biological control. J. Georgia Entomol. Soc. 19 (Second supplement):6-13. 1984.

Genetic Manipulation of Viruses to Enhance their Effectiveness in Insect Control

It should be possible, through genetic manipulation of viruses, to increase their virulence, especially in cases where they appear to be less virulent than normal. It should be possible to produce isolates of nuclear polyhedrosis virus (NPV) which require only one polyhedral inclusion body (PIB) to infect small larvae. It should be possible to shorten the time from infection to mortality and to increase virulence for larger larvae. Increasing virulence for larger larvae is important both in the field and in the commercial production of the virus.

It should be possible to expand the host range of some of the viruses so that a single virus can be used to control all or most of the lepidopterous pests on a given crop. This would be easier where most of the pests are in the same family, but it may be possible to produce a virus that will infect selected members of several families of Lepidoptera without being a direct threat to beneficial insects. A virus with an expanded host range would have three major advantages: First, it would cost the farmer less to apply one virus for a complex of pests than to apply two or three different viruses, one specific for each pest. Second, which ever pest was killed by the virus, it would produce inoculum that would help to protect the crop from any of the pests that were susceptible to the virus. Third, industry would be more interested in producing the virus because of the larger market.

1. Scientist's name, address, and telephone number:

Leslie C. Lewis
USDA-ARS
Corn Insects Research Unit
RR, Box 45B
Ankeny, IA 50021 Telephone 515-284-4758 FTS 862-4758

2. Location:

Same

3. Number and title of CRIS work unit:

3804-20240-004
Biological Control and Insect Pathology - Corn Insects

4. Approach Element and Problem Definitions:

Reduce losses - weeds, diseases, insects, nematodes in field crops. Current technologies are not adequate to protect - with maximum effectiveness and safety - against crop losses caused by plant diseases

5. Estimated SY's:

One

6. Objectives of research:

Determine the most efficacious pathogens and develop techniques to integrate these into a pest management system to suppress populations of the armyworm, black cutworm, and European corn borer.

7. Research priorities in your program:

The main thrust is to develop biological control components to integrate into a pest management system to suppress corn insects.

Priorities are (1) to determine ineractions and/or compatability of dual infections of pathogens and the compatability of pathogens with insect parasitoids; (2) determine the impact of reduced tillage (more crop residue) on the interseasonal transmission of insect pathogens; (3) develop techniques of initiating epizootics of insect diseases so that these epizootics will occur earlier in the growing season.

8. Progress of current research in solving problems:

Bacillus thuringiensis, chemical insecticides, and Nosema pyrausta act independently of each other and their effects are additive in terms of reducing larval populations. European corn borers infected with N. pyrausta will transmit the microsporidium to healthy insects via the frass under field conditions. N. pyrausta remains viable in corn borer frass within the corn plant during the winter. Black cutworms infected with sublethal levels of NPV from Rachiplusia ou will support the development of the parasitoid Bonnetia comta.

9. Significant research accomplishments in the past 3 years:

Treatments with combinations of NPV from Rachiplusia ou, Vairimorpha necatrix, and V. sp. can have additive, synergistic, or antagonistic effects on mortality of black cutworms

Demonstrated field efficacy of nuclear polyhedrosis viruses from Autographa californica and Rachiplusia ou against the European corn borer and the black cutworm.

Demonstrated field persistence of Nosema pyrausta and Vairimorpha necatrix.

Showed that the black cutworm, armyworm, and European corn borer are susceptible to β-exotoxin from Bacillus thuringiensis.

Demonstrated that neonate European corn borer larvae can be killed by the δ-endotoxin of B. thuringiensis without the presence of the spore.

10. Impact of research accomplishments on science and the general public:

Research with insect viruses was the first report of a virus being pathogenic to the European corn borer and the first confirmed report of a virus in the black cutworm.

Performed the basic and applied research to show the feasibility of using granular formulations of Bacillus thuringiensis to suppress populations of the European corn borer. This gives the hybrid seed corn producers a product nontoxic to detasselling crews.

The determination that δ-endotoxin can kill neonate corn borers makes it no longer necessary to protect the B. thuringiensis spore from ultraviolet light.

11. Obstacles to achieving objectives:

The main obstacles are lack of operating money for support help and facilities large enough to increase our research output.

12. Future lines of needed research and plan for implementation:

Basic research is needed on persistence of microbials in nature. More data is needed on virus-insect relationships. Our present knowledge is on infectivity and efficacy on extremely small field plots. Work is needed on the potential for vertical and horizontal transmission, as well as on dual infections of NPV and the natural infections of N. pyrausta.

Basic laboratory work on transmission and dual infections will be undertaken as soon as money and support help will permit.

13. Research facilities and personnel needs:

Dissecting and phase contrast scopes with photo micrography capabilities are needed. A positive flow hood and a dust-free microscopy room would be desirable. Additional insect incubation space isolated from the rearing facilities of the laboratory are needed.

Personnel needs could be met with hourly labor during peak times.

14. Extent of cooperation--names of persons and institutions:

Dr. J. V. Maddox, Economic Entomology, Natural History Survey, Urbana Illinois

Dr. R. E. Andrews, Jr., Department of Microbiology, Iowa State University

Dr. C. C. Beegle, USDA,ARS, Brownsville, TX.

15. Titles of publications for the last 3 years:

See attached.

Lewis, L. C. and Johnson, T. B. 1982. Efficacy of two nuclear polyhedrosis viruses against Ostrinia nubilalis in the laboratory and field. Entomophaga 27:33-38.

Johnson, T. B. and Lewis, L. C. 1982. Evaluation of Rachiplusia ou and Autographa californica nuclear polyhedrosis viruses in suppressing black cutworm damage to seedling corn in the greenhouse and field. J. Econ. Entomol. 75:401-404.

Johnson, T. B. and Lewis, L. C. 1982. Pathogenicity of two nuclear polyhedrosis viruses in the black cutworm, Agrotis ipsilon (Lepidoptera:Noctuidae). Can. Entomol. 114:311-316.

Lewis, L. C., Lublinkhof, J., Berry, E. C. and Gunnarson, R. D. 1982. Response of Ostrinia nubilalis (Lepidoptera:Pyralidae) infected with Nosema pyrausta to insecticides. Entomophaga 27:211-218.

Mohd-Salleh, M. B. and Lewis, L. C. 1982. Toxic effects of spore/crystal ratios of Bacillus thuringiensis on European corn borer larvae. J. Invertebr. Pathol. 39:290-297.

Lewis, L. C. 1982. Persistence of Nosema pyrausta and Vairimorpha necatrix measured by microsporidiosis in the European corn borer. J. Econ. Entomol. 75: 670-674.

Mohd-Salleh, M. B. and Lewis, L. C. 1982. Feeding deterrent response of corn insects to β-exotoxin of Bacillus thuringiensis. J. Invertebr. Pathol. 39: 323-328.

Lewis, L. C., Cossentine, J. E. and Gunnarson, R. D. 1982. Pathogenicity of Vairimorpha necatrix (Microsporidia:Nosematidae) against Ostrinia nubilalis (Lepidoptera:Pyralidae). Can. Entomol. 114:599-603.

Lewis, L. C. 1982. Present status of introduced parasitoids of the European corn borer, Ostrinia nubilalis (Hübner), in Iowa. Iowa State J. Sci. 56:429-436.

Lewis, L. C., Lublinkhof, J., Berry, E. C. and Gunnarson, R. D. 1982. Response of Ostrinia nubilalis (Lepidoptera:Pyralidae) infected with Nosema pyrausta to insecticides. Entomophaga 27:211-218.

Lewis, L. C. 1982. The use of granules and dusts to disseminate insect pathogens. Proceedings - International Colloquium on Invertebrate Pathol, pp 66-70. Brighton, United Kingdom.

Lewis, L. C., Cossentine, J. E. and Gunnarson, R. D. 1983. Impact of two microsporidia, Nosema pyrausta and Vairimorpha necatrix, in Nosema pyrausta and Vairimorpha necatrix, in Nosema pyrausta infected European corn borer (Ostrinia nubilalis) larvae. Can. J. Zool. 61:915-921.

Mohd-Salleh, M. B. and Lewis, L. C. 1983. Comparative effects of spore crystal complexes and thermostable exotoxins of six subspecies of Bacillus thuringiensis on Ostrinia nubilalis (Lepidoptera:Pyralidae). J. Invertebr. Pathol. 41:336-340.

Cossentine, J. E. and Lewis, L. C. 1984. Interactions between Vairimorpha necatrix, Vairimorpha sp., and a nuclear polyhedrosis virus from Rachiplusia ou in Agrotis ipsilon larvae. J. Invertebr. Pathol. 44:28-35.

1. Scientist's name, address, and telephone number:

Wm. H. McGaughey 913-776-2705
D. E. Johnson 913-776-2724

2. Location:

U. S. Grain Marketing Research Laboratory
1515 College Avenue
Manhattan, Kansas 66502

3. Number and title of CRIS work unit:

3420-20620-011 INTEGRATION OF MICROBIAL INSECTICIDES INTO STORED-GRAIN PEST MANAGEMENT SYSTEMS

4. Approach Element and Problem Definitions:

4.3.01.1

5. Estimated SY's:

1.6

6. Objectives of research:

Many insect pests that infest stored grain and processed cereal products are susceptible to microbial insect pathogens such as certain bacteria, viruses, and fungi. These microorganisms are selective in their insect pathogenicity, do not pollute the environment, and are safe to humans and other mammals. Our research with these organisms involves basic and applied studies of the structure, physiology, and mode of action of selected bacterial and viral insect pathogens. These studies include the use of Bacillus thuringiensis and granulosis virus to control the Indianmeal moth and other lepidopteran pests of stored grains; structure, toxicity, and biosynthesis of the entomocidal protein of B. thuringiensis; use of insect tissue culture for in vitro determination of molecular toxicity; and measurement of differential toxicity between various B. thuringiensis isolates.

7. Research priorities in your program:

1. Use of B. thuringiensis (BT) spores and crystals to control insect infestations in stored cereal grains.

2. Physiology of the toxic response to BT crystals in the gut of susceptible insects.

3. Natural resistance among field insect populations to BT.

4. Molecular biology of the granulosis virus of Plodia interpunctella.

8. Progress of current research in solving problems:

Control of Indianmeal moth (IMM) in grain bins with BT is effective and has been approved by EPA as an alternative treatment method to the use of malathion. Increased incidence of resistance among IMM in field populations, however, has led to studies documenting the prevalence (or increase) in natural resistance to BT. In vitro methods with insect tissue culture has led to alternative mortality bioassay methods, as well as the study of toxin physiology at the membrane level.

9. Significant research accomplishments in the past 3 years:

1. Effectiveness of surface application of BT in on-farm stored grain in bins, leading to EPA approval of method for insect control.

2. Determination of incidence of natural resistance to BT among field populations of IMM.

3. Development of methods for studying BT crystal toxic protein at molecular level using cultured insect cells.

4. Characterization of the granulosis virus absorption process to the membrane of the host cell using vertebrate erythrocytes as a model system.

10. Impact of research accomplishments on science and the general public:

EPA approval of BT use in grain bins has an immediate and useful effect upon public. Discovery and recognition of resistance to BT among field populations of IMM is first known instance of natural resistance to BT. Research with granulosis virus to date has been instrumental in determining the mode of infectivity and may help to design an artificial (in vitro) system for growing the virus outside of its natural host.

11. Obstacles to achieving objectives:

Lack of an adequate in vitro system for propagating granulosis virus.

12. Future lines of needed research and plan for implementation:

1. Growth of granulosis virus in cultured insect tissue cells. Traditional methods have all failed, so we plan to first encapsulate the virions in liposomes constructed from lipids extracted from insect cells in culture. These encapsulated virions will then be administered to insect tissue cells in culture.

2. If the above plan proves feasible, it would provide ample quantities of virus for large scale testing of control of IMM infestations in stored cereal grains and processed flours.

13. Research facilities and personnel needs:

U.S. Grain Marketing Research Laboratory

---Ample equipment already available.

---One additional SY for total commitment to granulosis virus research would be necessary.

14. Extent of cooperation--names of persons and institutions:

C. C. Beegle, ARS, Cotton Insects Research, Brownsville, Texas

H. Dulmage, ARS, Cotton Insects Research, Brownsville, Texas

R. Consigli, Kansas State University, Manhattan, Kansas

15. Titles of publications for the last 3 years:

See attached

McGaughey, W. H. and E. B. Dicke. Methods of applying Bacillus thuringiensis to stored corn for moth control. J. Econ. Entomol. 73:228-229. 1980.

McGaughey, W. H., E. B. Dicke, K. F. Finney, L. C. Bolte, and M. D. Shogren. Spores in dockage and mill fractions of wheat treated with Bacillus thuringiensis. J. Econ. Entomol. 73:775-778. 1980.

Johnson, D. E., and B. Freedman. Toxicity of Bacillus thuringiensis Spo^{-} Cr^{+} mutants for the European corn borer, Ostrinia nubilalis. Appl. Environ. Microbiol. 42:385-387. 1981.

Johnson, D. E. Toxicity of Bacillus thuringiensis entomocidal protein toward cultured insect tissue. J. Invertebr. Pathol.38: 94-101. 1981.

McGaughey, W. H. Evaluation of commercial formulations of Bacillus thuringiensis for control of the Indianmeal moth and almond moth (Lepidoptera: Pyralidae) in stored inshell peanuts. J. Econ. Entomol. 75:754-757. 1982.

Quinlan, J. K., and W. H. McGaughey. Fumigation of empty grain drying bins with chloropicrin, phosphine, and liquid fumigant mixtures. J. Econ. Entomol. 76:184-187. 1983.

McGaughey, Wm. H. Compatibility of Bacillus thuringiensis and Captan® when used in a mixture for treating stored grain for moth control. J. Econ. Entomol. 76(4):897-898. 1983

Johnson, D. E. and Wm. H. McGaughey. Insecticidal activity of spore-free mutants of Bacillus thuringiensis toward the Indianmeal moth and almond moth. J. Invertebr. Pathol. 43:156-159. 1984.

Johnson, D. E. and L. I. Davidson. Specificity of cultured insect tissue cells for bioassay of entomocidal protein from Bacillus thuringiensis. In Vitro 20:66-70. 1984.

Johnson, D. E. Selection for resistance to Bacillus thuringiensis δ-endotoxin in an insect cell line (Choristoneura fumiferana). Experientia 40:274-275. 1984.

1. Scientist's name, address, and telephone number:
Jean R. Adams
Insect Pathology Laboratory, PPI
344-3432

2. Location:
Beltsville, Md

3. Number and title of CRIS work unit:
1210-20261-005 Arthropod viruses: characterization, genetics, and replication in vivo and in vitro.

4. Approach Element and Problem Definitions:
2.4.09.1b Increased knowledge and technology for discovery and use of pathogens for pest control.

5. Estimated SY's:
0.75

6. Objectives of research:

7. Research priorities in your program:

8. Progress of current research in solving problems:
The rickettsial-like organism that is causing problems in many insect rearings was shown to be inhibited by penicillin. Sanitation and careful selection of rearing stock will help to eliminate it from insect colonies.

Two forms of virions involved in baculovirus infection have been demonstrated; the virions that are occluded in polyhedra and the virons that are involved in the spread of the infection. The specificity of the activity of the virions is one of the factors involved in an understanding of why insect viruses are specific in activity. The details of every step in the replication cycle are yet unknown.

9. Significant research accomplishments in the past 3 years:
1. Identification of Rickettsiella sp. infecting pecan weevils (Coop. with Dr. W. Neal, Mississippi State).
2. Established that rickettsial-like organism (RLO) isolated from about 15 insect species (lab reared and field collected) does not fit into present classification system in Bergey's Manual. This RLO is probably most closely related to rickettsia. It is very small, pleomorphic, highly infectious per os, transovum transmitted, and is inhibited by penicillin treatment of the diet. This organism is causing problems in many insect rearing facilities.
3. A small non-occluded virus isolated from crickets, in commercial rearings, is serologically related to a non-occluded virus isolated from field collected T. ni (Coop. with Dr. G. J. Tompkins).

10. Impact of research accomplishments on science and the general public:
Our research is providing data to: (1) improve the efficacy of insect pathogens; (2) establish that they are safe to use in microbial control of insects; and (3) better establish the role that microbial control is actually playing in controlling insect pests in nature.

11. Obstacles to achieving objectives:

1. Procurements procedures cause great delays in obtaining supplies and equipment needed.

2. Could use assistance of a 1040 student but we don't have funding for this.

12. Future lines of needed research and plan for implementation:

Investigation of sites of invasion and replication and spread of insect pathogens using immuno-cytochemical, electron microscopic techniques to better understand the specificity of insect viruses in the events that occur at the plasma membrane and within the cytoplasm and nuclear sites in the replication and spread of viral infections in vivo and in vitro. The protein A-gold, electron microscopic, immunocytochemical technique will be used to determine the ultrastructural localization of antigenic sites or receptor sites for viral attachment, fusion and activity. The pathway taken by specific insect viral proteins in infected cells and tissues will be studied. Specific antisera directed against purified and well characterized insect viral proteins will allow their detection at high resolution in infected cells and tissues (in Coop. with Drs. G. J. Tompkins and J. L. Vaughn).

13. Research facilities and personnel needs:

Data generator for STEM accessory
X-ray microanalysis accessory

14. Extent of cooperation--names of persons and institutions:

SBDL - Dr. Henis (Now returned to Israel)
PVL - Dr. A. Hadidi
FNCL - Dr. J. W. Neal, Jr.
IPL - Dr. G. J. Tompkins
Mississippi State - Dr. W. W. Neel

15. Titles of publications for the last 3 years:

1. Tompkins, G. J., J. L. Vaughn, J. R. Adams and C. F. Reichelderfer. 1981. Effects of propagating Autographa californica nuclear polyhedrosis virus and its Trichoplusia ni variant in different hosts. Environ. Entomol. 10:801-806.

2. Tompkins, G. J., J. W. Neal, Jr., J. Young and J. R. Adams. 1981. Eastern tent caterpillar control with nuclear polyhedrosis viruses in Maryland, 1981. Insecticide and Acaracide Tests. 7:215.

3. Goodwin, R. H., G. J. Tompkins, R. R. Gettig and J. R. Adams. 1982. Characterization and culture of virus replicating continuous insect cell lines from the bollworm, Heliothis zea (Boddie). In Vitro. 18:843-850.

4. Adams, J. R. and T. A. Wilcox. 1982. Scanning electron microscopical comparisons of insect virus occlusion bodies prepared by several techniques. J. Invertebr. Pathol. 40:12-20.

5. Faust, R. M., J. R. Adams, K. Abe, T. Iizuka and L. A. Bulla. 1982. Comparative morphology and size distribution of the parasporal crystals from various strains of Bacillus thuringiensis. J. Sericult. Sci. Japan 51:316-324.

6. Adams, J. R. 1983. Electron microscopic examinations of virin ENSH and Gypchek NPV In "A Comparison of the US(Gypchek) and USSR (Virin-ENSH) preparations of the nuclear polyhedrosis virus of the gypsy moth L. dispar. Ignoffo, Martignoni, and Vaughn, eds. Publ. Am. Soc. Microbiol., Wash. D.C. pp. 11-20.

1. Scientist's name, address, and telephone number:
Edward M. Dougherty
Insect Pathology Laboratory, PPI
301-344-3692

2. Location:
Beltsville, MD.

3. Number and title of CRIS work unit:
1210-20261-005 Arthropod Viruses: Characterization, genetics and replication *in vivo* and *in vitro*.

4. Approach Element and Problem Definitions:

2.2.01.01f,h,i	2.4.09.01b
2.4.01.04a,b	2.4.01.2a,b,c
2.2.05.01b	

Research planning workshop on fundamental insect biology sections.

2.2.01.10	2.2.05.1b
2.2.01.1g	2.4.01.1b
2.2.01.1i	2.4.01.2c

Finally sections E_2 and E_3 are of interest from the ARS research planning conference on biological control.

5. Estimated SY's:
1

6. Objectives of research:
Overall objective is to gain basic knowledge about insect viruses which will enhance their effective use in IPM programs. The approach to this objective has consciously evolved over the last decade. Rather than persue traditional biocontrol programs I am studying the molecular events responsible for empiracle observations from field work and from laboratory *in vivo* studies in order to generate the basic knowledge needed to intelligently change the genetic make up of the virus. In addition, I am also aware of the tremendous potential of the viruses to serve as expression vectors for carrying future novel control agents directly to species specific targets. Although many years from practical application, the biological breakthroughs of the last decade make this type of approach possible and will probably produce the biocontrol results once thought possible with naturally occurring viruses.

7. Research priorities in your program:
1. Retention of trained personnel.
2. Adequate funding to persue Dr. Kinney's mandate of excellence.
3. Future emphasis will be on the LdMNPV system. Currently efforts are being made to generate the physical map of the genome of several strains of LdMNPV. The genetic libraries resulting from these maps will be used to find virulence factors by trying to restore virulence to avirulent strain by coinfection with cloned fragments of virulent strains. In addition, several genes will be cloned in the virus and characterized. Deletion mutations will be made and cloned virulent genes inserted to demonstrate their function. One such source will hopefully come from collaboration with the Insect Reproduction Lab

Pla, Ph

research Program

approach possible and

possible with naturally occurring

efforts in our program.

of trained personnel.

to persue Dr.

be on the

nerate the

libraries resulting from these

lying to restore

fragments of

characterized

genes inserted to determine their function.

From collaboration with the

in an effort to clone two insect hormones of interest. Finally, host range studies of LdMNPV and other baculoviruses especially hypervirulent baculoviruses will be made in semi-permissive systems to study what blocks have to be overcome to produce natural hypervirulent strains of virus in the gypsy moth. This portion of work will involve approximately 90% of my efforts over the next three years. The remaining time allotment will be used with cooperative efforts with other scientists both USDA and non-USDA and in unforeseen research problems which can not be currently anticipated. The overall effort is molecular in nature with few exceptions, however, it is also a directed program aimed at finding out weaknesses of virus as control agents and correcting these or adding to already strong points about viruses to produce a useful biocontrol agent.

8. Progress of current research in solving problems:

Progress is better than adequate, with potential for good progress with long term committments of current finances. With the new technologies available and which are being developed in my lab excellent progress could be made with modest increases in funding and the freedom to spend this funding. The total amount of progress made and anticipated is excellent when funding and personnel committments are compared to several of my colleagues in non-USDA research programs both in other government agencies and in the private sector.

9. Significant research accomplishments in the past 3 years:

1. Described the viremia of a granulosis virus of the cabbage looper.
2. Although unsuccessful in achieving in vitro virus replication, developed new cell lines of the cabbage looper.
3. Characterized the reported longevity of granulosis virus infected insects and showed that a hormonal depression caused by GV was responsible.
4. Developed a plaque assay for gypsy moth NPV which allows for initiation of genetic studies and quantitation of virus.
5. Showed the presence of genotypic variants and differential virulence of these isolates in LdMNPV.
6. Showed the effect of diet on virulence of LdMNPV.
7. Characterized the temporal sequence of macromolecular synthese of LdMNPV in a permissive in vitro system.
8. Characterized the replication of a hypervirulent baculovirus in a semi-permissive in vitro L. dispar system.

10. Impact of research accomplishments on science and the general public:

The general public does not feel any impact from studies reported nor will they for several years. The goal of this project, modulation of gypsy moth populations with LdMNPV, is a multi year basic research problem necessary to LdMNPV and to many other biological control agents. The impact of research accomplishments on science is starting to become apparent. Results from my program the last three years have been communicated to most labs currently involved in LdMNPV research. As this program progresses there have been several recent active scientific investigators from USDA and academia visit my lab and have either shown interest in cooperating or coordinating research to resolve potential conflict. Several manuscripts now in preparation will further inform the scientific community of the progress being made with the LdMNPV system. As the quality and quantity of research on LdMNPV increases we will be able to integrate more knowledge from other virus and baculovirus programs into our own.

11. Obstacles to achieving objectives:

Lack of permanent technical assistance has been extremely crippling in developing a state of the art research program. A lack of a nucleus of research scientists devoted to the molecular aspect of insect virology has also hampered the efficiency and variety of approaches possible for solving problems associated with insect viruses. Both problems necessitated getting outside money to build my own program. Problems encountered in this area are a lack of long term committment from project managers (more than one year) and recently questioning of how the money is spent acquiring needed resources (long termed trained or trainable people) through cooperative agreements with universities. Finally the amount of funding both hard and soft money is not enough to carry out state of the art research programs envisioned by Director Kinney.

12. Future lines of needed research and plan for implementation:

As a whole ARS needs a much greater molecular approach to their biocontrol program. Until one finds out why things don't work (through basic research) only missdirected research dollars and luck will produce an occassional biological control agent. My own goal is to continue to press for funds to try to establish a nucleus of interested molecular oriented virolgists at IPL and to cooperate with several programs of excellence in this area such as the Insect Virus Lab at Boyce Thompson Institute.

It is obvious from the results of the use of viruses that they have not provided satisfactory efficacy. The difference between desired results and actual results are extremely small in many instances. Thus a small increase in efficacy either in application, persistance or virulence is all that is needed for several viral pesticides to be successful. The approaches outlined in 6 and 7 of this questionnaire will be followed.

Although these approaches are immediate in nature the greatest potential use of insect viruses which has been totally overlooked by ARS but not in several labs outside of ARS is the use of these viruses to serve as expression vectors for genetic elements capable of regulating insect populations. My own goals do not overlook such possibilities with LdMNPV.

13. Research facilities and personnel needs:

In order to persue that portion of my research involving biotechnology approximately $40,000 in equipment is needed over the next three years. In order to make reasonable progress in stated research plans an increase in soft money or base funds from approximately $30,000/yr to $50,000/yr is needed for hiring and retaining trained personnel. As I have related to the ARS gypsy moth project leader this is an extremely reasonable figure when compared to other research institutes both private and public outside ARS. Acquisition of these personnel and increased cooperation with other groups of excellence as my own program improves will satisfy the needs for a nucleus of molecular oriented individuals.

14. Extent of cooperation--names of persons and institutions:

Dr. Martin Shapiro ARS, Otis AFB, Mass. Improved virulence of LdMNPV.

Dr. Kathleen Shields, FS, Hampdon, Conn. LdMNPV electron microsopy and *in vivo* and *in vitro* replication.

Dr. William McCarthy, Penn State Univ., College Park, Pa. LdMNPV genome mapping and *L. dispar* cell cultures.

Dr. Ronald Weiner, Univ. of Md., College Park, Md. LdMNPV replication and genetic engineering.

Dr. Allan Wood, Cornell Univ., Ithaca, NY. LdMNPV genome mapping and viral latency.

Dr. Thomas Kelley, and Dr. Peter Masler, ,ARS, Beltsville, Md. Effect of viruses on hormone production in vivo. Cloning of insect neuro peptides.

Dr. George Cantwell, ARS, Beltsville, Md. In vitro assay of toxins and microbial control agents.

15. Titles of publications for the last 3 years:

1. S. A. Weiss, G. C. Smith, S. S. Kalter, J. L. Vaughn, and E. M. Dougherty. Improved replication of Autographa californica nuclear polyhedrosis virus in roller bottles: Characterization of progeny virus. Intervirology 15, Issue 3-4, 1981.

2. E. M. Dougherty, R. M. Weiner, J. L. Vaughn and C. F. Reichelderfer. Physical factors that affect in vitor Autographa californica nuclear polyhedrosis virus infection. Applied and Environm. Micro. 41:1166-1172. 1981.

3. E. M. Dougherty. Baculovirus in vitro quantification. US USSR Joint Commission on Scientific and Technical Cooperation. U.S. Working Group on Microbiology (01.07): Project 5 Microbiological Control of Insect Pests. Microbial Control of Pest of Agricultural Crops. Clearwater, Fl. 1981.

4. E. M. Dougherty, G. E. Cantwell, and M. Kuchinski. Biocontrol of the greater wax moth Galleria mellonella (Lepidoptera: Pyralidae) using a homologous embedded nuclear polyhedrosis virus. J. of Econ. Entom. 75:675-679. 1982.

5. Weiss, S. A., J. L. Vaughn, T. Orr, G. C. Smith, E. M. Dougherty and S. S. Kalter. Quantiative measurements of oxygen consumption in insect biotechnology. 24:1145-1154. 1982.

6. Weiss, S. A., G. C. Smith, S. S. Kalter, J. L. Vaughn, E. M. Dougherty, and G. J. Tompkins. Effect of aluminum chloride and zinc sulfate on Autographa californica nuclear polyhedrosis viru (ACNPV) replication in cell culture. In Vitro. 18:937-944. 1982.

7. Tompkins, G. J., J. V. Linduska, E. M. Dougherty and J. M. Young. Control of Lepidoptera larvae on collards with nuclear polyhedrosis virus and Bacillus thuringiensis, 1982. In "Insecticide Acaricide Tests", Entom. Soc. of America. 7:119-120. 1983.

8. E. M. Dougherty and C. F. Reichelderfer. Characterization of the viremia of a granulosis virus of Trichoplusia ni. (Abstract) XIV Annual Meeting. Soc. for Invertebr. Pathol., Bozeman, MT. 1981.

9. E. M. Dougherty, R. M. Weiner, J. L. Vaughn and C. F. Reichelderfer. Rifampin inhibition of the occluded virus form of a nuclear polyhedrosis virus. Antimicrobial Agents and Chemotherapy. 22:527-530. 1982.

10. E. M. Dougherty, M. Shapiro, J. R. Adams and R. Rochford. Partial characterization of several geographical and plaque purified isolates of a multiple embedded nuclear polyhedrosis virus of the gypsy moth Lymantria dispar (MLdNPV) (Abstract) Soc. for Invertebr. Path., Univ. Park, PA. 1982.

11. E. M. Dougherty. Characterization and improvement of a multiple embedded nuclear polyhedrosis virus of the gypsy moth Lymantria dispar (Report). National Gypsy Moth Review Meeting, Harrisburg, PA. Dec. 9, 1982.

12. E. M. Dougherty. Current status of Lymantria dispar nuclear polyhedrosis virus as a control agent for the gypsy moth Lymantria dispar. IV International Conference on Compartive Virology. Banff, Canada. Oct. 17-22, 1982.

13. J. T. McClintock, E. M. Dougherty, R. M. Weiner. In vitro replication of a multiple embedded nuclear polyhedrosis virs of Lymantria dispar, the gypsy moth in a homologous cell line (Abstract). 83rd Annual Meeting American Society for Microbiology, New Orleans, LA. Mar. 6-11, 1983.

14. E. M. Dougherty, J. T. Kelly. Hormonal depression of Trichoplusia ni larvae by infection with its homologous granulos virus (Abstract). 83rd Annual Meeting American Society for Microbiology, New Orleans, LA. Mar. 6-11, 1983.

15. S. A. Weiss, D. PAPLOW, J. L. Vaughn, E. M. Dougherty. Biotechnological aspects of a large scale process for insect cells and baculoviruses (Abstract). International Conference on Invertebr. Tissue Cult. St. Augustine, FL. June 5-10, 1983.

16. G. E. Cantwell, E. M. Dougherty and W. W. Cantelo. Activity of the β-exotoxin of Bacillus thuringiensis var. thuringiensis against the Colorado potato beetle (Coleoptera: Chrysomelidae) and bacterial mutagenic responses as determined by the Ames Test. Environ. Entomology, 12:1424-1427. 1983.

17. E. M. Dougherty. A comparison of the Virin-Ensh and Gypchek commercial preparations of the multiple embedded nuclear polyhedrosis virus of the gypsy moth, Lymantria dispar (LdMNPV) utilizing restriction endonuclease analysis (REN). Report of the Joint US/USSR Working Group for Scientific and Technical Cooperation on the Production of Substances by Microbiological Means. Project V Microbiological Preparations for Control of Insect Pests. 21-30 pp. 1983.

18. E. M. Dougherty, M. Shapiro and K. Shields. ABS Eastern Branch Entomological Society of America. 55th Annual Meeting, Providence, R.I. Sept. 18-21, 1983. Effect of foliage on virulence and replication of a mulitply embedded nuclear polyhedrosis virus of the gypsy moth Lymantria dispar (Lepidoptera: Lymantriidae).

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[illegible] 21, 1983. [illegible] on virulence and
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19. R. Rochford, E. M. Dougherty, and D. Lynn. Establishment of a cell line from embryos of the cabbage looper, Trichoplusia ni (L.). In Vitro (New Cell Line Section). 1984.

20. Vaughn, J. L. and Dougherty, E. M. The replication of baculoviruses. In Viral Insecticide for Biological Control, (K. Marmorosch, K. E. Sherman, ed.). Academic Press, New York. 1984. (In Press)

1. Scientist's name, address, and telephone number:
Dwight E. Lynn
USDA,ARS,NER,Insect Pathology Laboratory, PPI
BARC-W, Beltsville, MD (301)344-4328

2. Location:
Beltsville Agricultural Research Center, Beltsville, Md.

3. Number and title of CRIS work unit:
1210-20261-005 Arthropod viruses: characterization, genetics, and replication in vivo and in vitro.

4. Approach Element and Problem Definitions:
2.2.01.1h In vitro culture of insect cells.
2.4.09.1b Discovery and efficient use of pathogens for pest control.

5. Estimated SY's:
1

6. Objectives of research:
To develop in vitro systems (insect cell cultures for studying viruses and other insect pathogens. This is an essential step for developing viruses as biological control agents because cell cultures can be used to: (1) select virus strains; (2) measure pathogenicity; (3) identify viruses; (4) for production; and is an essential tool for any genetic manipulations of obligate pathogens such as viruses.

7. Research priorities in your program:
1. Development of new cell cultures from important insect species (especially Coleoptera, Hymenoptera, and Lepidoptera) and determine their utility for replicating pathogens.

2. Improve media for culturing insect cells and producing viruses. This includes optimizing nutrients and determining the necessity of other growth promoting factors (such as hormones) important in virus replication. This research centers on the model system of Autographa californica nuclear polyhedrosis virus in lepidopteran cell cultures.

8. Progress of current research in solving problems:
1. Cell lines known to be responsive to the insect hormone 20-hydroxyecdysone are being tested for effects of hormone treatments on virus replication.

2. Diabrotica undecimpunctata cell lines are being used to grow fastidious spiroplasmas from Colorado potato beetle.

3. Mammalian growth factors (epidermal G.F., fibroblast G.F., etc.) are being tested in low-serum media as serum substitutes.

4. Field collected D. undecimpunctata viruses are being tested in cell cultures.

5. A new cell line from the hymenopteran parasite, Trichogamma pretiosum has been developed.

9. Significant research accomplishments in the past 3 years:

1. New cell lines established from:
   a. D. undecimpunctata, the southern corn rootworm (two new lines which are only the second successful development of beetle cell cultures).
   b. Trichoplusia ni embryos.

2. An insect growth factor was discovered in lepidopteran hemolymph which may give important new information on the control of development in insects and improve the understanding of conditions necessary for in vitro growth of cells.

10. Impact of research accomplishments on science and the general public:
The new cell lines have provided tools for a number of scientists in several fields of research, including several areas of pathology (virology, mycoplasmology, bacteriology and protozoology), developmental biology, and radiation biology.

11. Obstacles to achieving objectives:
1. Thus far, no cell/virus systems have been developed for Diabrotica or closely related species. Most known viruses from these insects are less likely candidates for biological control than those discovered from lepidopteran insects.

2. Granulosis viruses cannot generally be grown in vitro. Only one cell line from insects developed after extensive testing has been found capable of supporting granulosis virus replication. This is a necessary condition for developing granulosis viruses as biological control agents.

12. Future lines of needed research and plan for implementation:
1. Develop assay methods for identifying specific cell types (i.e., epithelial, nerve, fat body, etc.) in culture. A BARD proposal has been submitted which includes plans for using monoclonal antibodies to intermediate filaments in immunofluorescent techniques.

2. Develop HPLC technology for measuring nutrient utilization in cell culture. Initially this will be used to study and optimize amino acids in lepidopteran cell cultures for virus replication. The necessary equipment is being modified for this purpose.

3. Develop mutant cell lines for use in hybridization techniques with somatic cells from insects. Initial efforts will be to isolate HAT sensitive cell lines such as have been developed with mammalian cell cultures. If successful, the known fusion techniques will be used to develop hybrids between continuous insect cell lines and fat body or midgut tissue for use in granulosis virus research.

13. Research facilities and personnel needs:
Current facilities are adequate with minor equipment additions or modifications. Additional technical personnel would increase productivity.

14. Extent of cooperation--names of persons and institutions:

Dr. Herbert Oberlander	Insect Attractants Behavior and Basic Biology Lab, Gainesville, FL
Dr. Steven Ferkovich	Insect Attractants Behavior and Basic Biology Lab, Gainesville, FL
Dr. Akey Hung	Beneficial Insects Introduction Lab, IIBIII, BARC
Dr. Mark Feldlaufer	Insect Physiology Lab, PPI, BARC
Dr. Kevin Hackett	Insect Pathology Lab, PPI, BARC
Dr. Truman Clark	Insect Pathology Lab, PPI, BARC
Dr. Robert Whitcomb	Insect Pathology Lab, PPI, BARC
Dr. Edward Dougherty	Insect Pathology Lab, PPI, BARC

15. Titles of publications for the last 3 years:

1. Lynn, D. E., Miller, S. G., and Oberlander, H. Development of a cell line from lepidopteran wing imaginal discs: Induction of newly synthesized proteins by 20-hydroxyecdysone. Proc. Natl. Acad. Sci. USA 79: 2589-2593. 1982.

2. Oberlander, H., and Lynn, D. E. Morphogenesis in insect tissue culture. Advances in Cell Culture 2: 237-265. 1982. (Maramorosch (ed), Academic Press, N.Y.)

3. Oberlander, H., Lynn, D. E., and Leach, C. E. Inhibition of cuticle formation in cultured imaginal discs: Effects of colcemid and vinblastine. In Vitro 18: 29. 1982. (Abstract)

4. Lynn, D. E. Development of a cell line from the coleopteran insect, Diabrotica undecimpunctata. In Vitro 19: 262. 1983. (Abstract)

5. Lynn, D. E., and Oberlander, H. The establishment of cell lines from imaginal wing discs of Spodoptera frugiperda and Plodia interpunctella. J. Insect Physiol. 29: 591-596. 1983.

6. Lynn, D. E., Boucias, D. G., and Pendland, J. C. Nuclear polyhedrosis virus replication in epithelial cell cultures of Lepidoptera. J. Invertebr. Pathol. 42: 424-426. 1983.

7. Oberlander, H., Lynn, D. E., and Leach, C. E. Inibition of cuticle production in imaginal discs of Plodia interpunctella (cultured in vitro): Effects of colcemid and vinblastine. J. Insect Physiol. 29: 47- 53. 1983.

8. Lynn, D. E., and Oberlander, H. Characteristics of cell lines derived from imaginal discs of three species of Lepidoptera. Invert. T. C. Conf. Proceedings. 1984. (In Kurstak, Maramorosch, and Oberlander, eds., in press)

9. Lynn, D. E., and Stoppleworth, A. Established cell lines from the beetle, Diabrotica undecimpunctata (Coleoptera: Chrysomelidae). In Vitro 20: 365-368. 1984.

10. Rochford, R., Dougherty, E. M., and Lynn, D. E. Establishment of a cell line from embryos of the cabbage looper, Trichoplusia ni (L.). In Vitro 1984. (in press)

11. Streett, D. A., and Lynn, D. E. Nosema bombycis replication in a Manduca sexta cell line. J. Parasitol. 70: 452-454. 1984.

12. Hackett, K. J., and Lynn, D. E. Cell assisted growth of a fastidious spiroplasma. ASM Abs. 85 1985. (accepted for presentation at 1985 meeting)

13. Lynn, D. E., Oberlander, H., and Ferkovich, S. M. Induction of vesicle formation in a cell line derived from imaginal discs. In Vitro. (in press)

1. Scientist's name, address, and telephone number:
George Tompkins
Insect Pathology Laboratory, PPI
344-4325

2. Location:
Beltsville, Md

3. Number and title of CRIS work unit:
1210-20261-005 Arthropod viruses: characterization, genetics, and replication *in vivo* and *in vitro*.

4. Approach Element and Problem Definitions:
2.2.4.09.1b Increased knowledge and technology for the discovery and use of pathogens.

5. Estimated SY's:
1

6. Objectives of research:
1. Determine effects of propagation of insect viruses in alternate hosts and determining DNA changes associated with the virulence enhancement.

2. Field evaluation studies of most promising viruses with aid of shade protectants and addition of microsporidian of bacterial agents to determine most effective methods of obtaining desired level of control of insect target pests on cole crops.

3. To set up screening tests for determining effects of biocontrol agents on nontarget organisms and ways of diagnosing for these organisms.

7. Research priorities in your program:
1. Create more virulent strains of insect viruses.

2. Formulation of optimum amount of virus to be applied and determine effects of protective agents in actual field use.

3. Determine DNA changes associated with host production changes which alter virulence.

4. Determine factors and antigens associated with host range of viruses and attachment to membranes.

5. Determine ecological factors which enable better useage of biocontrol agents in an integrated pest control program.

8. Progress of current research in solving problems:
1. Have established means of determining safety of biocontrol agents for EPA testing in shrimp.

2. Have found host producing most virulent virus for control of several insect pests.

3. Have determined value of UV protective agents under actual field conditions and also the mixing of two or more biocontrol agents in same application.

9. Significant research accomplishments in the past 3 years:
1. Have confirmed that insect nuclear polyhedrosis viruses do change regarding DNA patterns with corresponding changes in virulence when produced in alternate hosts and cell lines. The DNA patterns with different enzymes are significantly different from the original wild type virus population.

2. Have shown that the current marketed B. t. formulation does not yield as effective control as a different B. t. formulation does.

10. Impact of research accomplishments on science and the general public:
Since first demonstrating that the virulence of nuclear polyhedrosis viruses is altered when passaged in alternate hosts, many other researchers have been applying this technique for practical application on target plants. An advantage of virus altered this way over plaque purified virus is that it can change when exposed to another susceptible insect species. The impact for future useage may be that of decreased costs for production and biocontrol programs.

11. Obstacles to achieving objectives:
Primary obstacles are within our system of technician promotions, peer review panel system for scientists, and the forever changing system of not knowing if money will be available for needed equipment to continue basic research programs. If required equipment is not available then the moral deteriorates because one can't compete with other organizations that are better equipped and that have a more stable technical support system than we have. You can't keep good technical help if they can't be promoted beyond GS-7 when they can go to NIH, Walter Reed or any number of other organizations and get GS-9 positions immediately and in one year be promoted to GS-11.

12. Future lines of needed research and plan for implementation:
Enzyme research is needed to determine genetic resistance in insect populations. Also ELIZA system for better serological evaluation of viral receptor site research is needed to do this. Management within the lab for this as well as technical help situation has to improve to compete with university or other research labs.

13. Research facilities and personnel needs:
A continuous supply of cells for serology research. A thoroughly trained technician with expertise in general immunology, DNA extraction procedures, REN fragmentation, electrophoresis knowledge and with general training in monoclonal antibody and ELIZA techniques.

14. Extent of cooperation--names of persons and institutions:
Dr. John Couch, EPA, Sabine Island, Gulf Breeze, Florida.
Dr. James Linduska, U.Md., Veg. Res. Farm, Salisbury, Md.
Dr. Fred Preiss, MGM, Minneapolis, Minnesota.
Dr. C. F. Reichelderfer, U. Md., College Park, Md.

15. Titles of publications for the last 3 years:

1. Tompkins, G. J., J. J. Linduska, E. M. Dougherty and J. Young. 1982. Control of Lepidoptera on collards with nuclear polyhedrosis viruses and Bacillus thuringiensis, 1981. ESA Insect. and Acar. Tests. 7:84-85.

2. Tompkins, G. J., J. R. Adams and J. M. Young. 1982. Effects of propagating multiple embedded nuclear polyhedrosis viruses in alternate hosts. IIIrd International Colloq. on Invert. Pathol., Brighton, UK. p. 79. (Abstract)

3. Tompkins, G. J., J. W. Neal, Jr., J. Young and J. R. Adams. 1982. Eastern tent caterpillar control with nuclear polyhedrosis viruses in Maryland. Insecticide Acaricide Tests. 7:215.

4. Goodwin, R. H., G. J. Tompkins, R. R. Gettig and J. R. Adams. 1982. Characterization and culture of virus replicating continuous insect cell lines from bollworm, Heliothis zea (Boddie). In Vitro 18:843-850.

5. Adams, J. R., C. C. Beegle and G. J. Tompkins. 1982. Pathogens recently isolated from insects in insect mass rearing facilities. IIIrd International Colloq. on Invert. Pathol., Brighton, UK. p. 124 (Abstract)

6. Adams, J. R., C. C. Beegle and G. J. Tompkins. 1982. A chlamydial-like organism isolated from insects in insect mass rearing facilities. 40th Ann. Proc. Electron Microscopy Sec. America., Wash. D.C. pp.316-317. (Abstract)

7. J. A. Couch, S. M. Martin, G. Tompkins and J. Kinney. 1984. A simple system for the preliminary evaluation of infectivity and pathogenesis of insect virus in a non-target estuarine shrimp. J. Invert. Pathol. 43:351-357.

8. G. J. Tompkins, J. J. Linduska, J. M. Young and E. M. Dougherty. 1983. Control of the cabbage looper and imported cabbageworm on collards with baculoviruses and B. t., 1983. Acaricide and Insecticide, ESA.

9. G. J. Tompkins., J. L. Vaughn, J. R. Adams and C. F. Reichelderfer. 1981. Effects of propagating Autographa californica nuclear polyhedrosis virus and its Trichoplusia ni variant in different hosts. Environ. Entomol. 10:801-806.

1. Scientist's name, address, and telephone number:
James L. Vaughn
Insect Pathology Laboratory, PPI
344-3688

2. Location:
BARC-W, Beltsville, Md.

3. Number and title of CRIS work unit:
1210-20261-005 Arthropod viruses: characterization, genetics, and replication in vivo and in vitro.

4. Approach Element and Problem Definitions:
2.2.01.1h In vitro culture of insect cells.
2.4.09.1b Discovery and efficient use of pathogens for pest control.

5. Estimated SY's:
1

6. Objectives of research:
1. Develop an understanding of the physiological mechanisms controlling the in vitro infectivity of the occluded form of the nuclear polyhedrosis viruses using the broad range Autographa californica nuclear polyhedrosis virus as a model system.

2. Develop serological methods for determining relationships between viral isolates.

3. Identify specific polypeptides that control virus attachment to host cells using serological analysis.

7. Research priorities in your program:
1. Identify the physiological factors that are required for the effective use of the occluded form of the virus in cultured cells.

2. Develop an in vitro system for the efficient, quantitative infection of insect cells with the occluded viruses.

3. Determine the serological relationships of several cloned strains of nuclear polyhedrosis viruses normally associated with Pieridae as a model system.

4. Identify specific polypeptides involved in virus neutralization.

8. Progress of current research in solving problems:
In vitro studies of the nuclear polyhedrosis viruses have required the use of the nonoccluded form of the virion. Thus, cell cultures cannot be used to monitor or standardize biocontrol products. Also, continued use of this form either in vitro or in vivo results in the selection of a variant that no longer produces polyhedra, the form used in biocontrol. I have developed methods for releasing the occluded virion from the polyhedra that give reproducable, infections in cell culture. However, based upon either the number of virus particles or of polyhedra per infectious unit the assay is not yet sensitive enough to be of practical value. It does allow for the maintenance of virus stocks in cell culture using occluded virions as

the inoculum. This avoids the selection of the non-polyhedron form and the possibility of contamination that frequently occurs when insects must be used to regenerate the stock.

9. Significant research accomplishments in the past 3 years:
1. Development of a 3 liter experimental cell culture system for the production of polyhedrosis viruses. This work was carried out in cooperation with Stefan Weiss at the Southwest Foundation for Education and Research, San Antonio, Texas. It was an extension of the studies begun here on the large volume culture of insect cells and is the most efficient system for the in vitro production of polyhedrosis viruses. The cooperative study was funded for 1½ years by ARS funds from the Administrators Reserve and for the last 3 years by a NSF grant to Stefan Weiss.

2. Development of a repeatable, effective method for releasing occluded virions from polyhedra thereby avoiding both the continuous use of the nonoccluded form and the need to regenerate the stock in insects.

10. Impact of research accomplishments on science and the general public:
1. Development of methods for the in vitro culture of the gypsy moth virus provided a contamination-free virus stock for use in production of this virus for field testing. Insect prepared inocula used in the past had often resulted in contaminated production runs that had to be rejected because they did not meet quality control standards.

2. Development of a cell line from Spodoptera frugiperda was the first cell line from an agriculturally important pest and is one of the 2 cell lines most frequently used in research on polyhedrosis viruses around the world.

3. Pioneering studies on the large volume culture of insect cells established the feasibility of cell culture for virus production and has lead to increasingly efficient in vitro systems that will eventually replace the insect methods presently used.

11. Obstacles to achieving objectives:
Time - Administration consumes increasingly higher percentages of my time, making it difficult to develop expertise in new methodologies. It is therefore essential to have good support help which I do have at the present time.

12. Future lines of needed research and plan for implementation:
Plans are to eventually identify those polypeptides that are required for attachment and penetration of the polyhedrosis virus into the permissive host cell. This process will be investigated using the virus inhibition tests that we have developed and a series of monoclonal antibodies specific for viral envelop peptides and capable of blocking attachment and penetration. The individual polypeptides will be isolated by gel electrophoresis and identified with the monoclonal antibodies by western blot analysis. This phase of the study will be done in collaboration with G. J. Tompkins in this laboratory. A. californica NPV will be used as a model system.

The genes that control the production of these polypeptides will be identified from gene maps being published by other laboratories. The feasibility of altering the host range of the viruses will then be studied by using genetic engineering procedures. This study will be done in collaboration with E. M. Dougherty of this laboratory.

13. Research facilities and personnel needs:
This work will require funds and equipment to establish a monoclonal antibody production facility at IPL. Although the use of such a facility is described here in relation to this particular research the facility would be used by other Laboratory scientists for the logical development of several projects in bacterial and spiroplasma research as well as the virus research.

14. Extent of cooperation--names of persons and institutions:
Mr. Stefan Weiss, Southwest Foundation for Education and Research.
Dr. M. E. Martignoni, USDA Forest Service, Corvallis, OR.
Dr. Carlo M. Ignoffo, USDA, ARS, BCIRL, Columbia, MO.

15. Titles of publications for the last 3 years:

1. Weiss, S. A., Peplow, D., Kalter, S. S. and Vaughn, J. L. Dissociation of insect cell cultures by pancreatin. In Vitro 18: 298. 1982. (Abstract)

2. Weiss, S. A., Smith, G. C., Vaughn, J. L., Dougherty, E. M. and Tompkins, G. J. Effect of aluminum chloride and zinc sulfate on Autographa californica nuclear polyhedrosis virus (ACNPV) replication in cell culture. In Vitro 18: 937-944. 1982.

3. Vaughn, J. L. and Stone, R. D. In vitro infectivity of the (VIRIN-ENSh) strain of the Lymantria dispar nuclear polyhedrosis virus. In (C. M. Ignoffo, M. E. Martignoni, and J. L. Vaughn, ed.) A comparison of the US (Gypchek) and USSR (VIRIN-ENSh) preparations of the nuclear polyhedrosis virus of the gypsy moth, Lymantria dispar. Microbiological Control of Insect Pests, of the US/USSR Joint Working Group on the Production of Substances by Microbiological Means. 32-37 pp. Am. Soc. Microbiol., Washington, D. C. 1983. (Project V-01.0705)

4. Weiss, S. A., Peplow, D., Smith, G. C., Vaughn, J. L. and Dougherty, E. M. Biotechnical aspects of a large-scale process for insect cells and baculoviruses. Proc. 6 Internat. Confer. Invertebr. Tissue Cult., St. Augustine, FL. 1983. (In Press)

5. Whitcomb, R. F., Clark, T. B. and Vaughn, J. L. Pathogenicity of mycoplasmas for arthropods and its possible significance in biological control. Methods in Mycoplasmology 2: 361-367. 1983.

6. Samish, M., Louloudes, S., Vaughn, J. L., Kurtti, T. J. and Munderloh, U. Promotion of tick cell growth by proline and fractions from tick eggs. Inter. J. Parasitol. 1984. (In Press)

7. Stone, R. D. and Vaughn, J. L. Selection of a pH indicator for the culture of insect cells. In Vitro 20: 270-271. 1984. (Abstract)

8. Vaughn, J. L. and Dougherty, E. M. The replication of baculoviruses. In (K. Marmorosch, K. E. Sherman, ed.) Viral Insecticide for Biological Control, Academic Press, New York. 1984. (In Press)

9. Vaughn, J. L., Zhu, G. K. and Stone, R. D. The infection of established insect cell lines with occluded virus of a nuclear polyhedrosis virus. Proc. 6 Internat. Confer. Invertebr. Tissue Cult., St. Augustine, FL. 1984. (In Press)

10. Weiss, S. A. and Vaughn, J. L. Chapter III. Cell culture methods for large-scale propagation of baculoviruses. In (R. G. Granados, B. Federici, ed.) The Biology of Baculoviruses, Practical Application for Insect Control. CRC Press, Boca Raton, FL. 1984. (In Press)

11. Vaughn, J. L. Insect tissue culture: Techniques and development. In (E. Kurstak, ed.) Techniques in Cell Biology. Elsevier Sci. Publ, Biomed Div., Amsterdam. 1985. (Book Chapter (In Press))

1. Scientist's name, address, and telephone number:

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USDA-ARS
Building 1398
Otis ANGB, MA 02542

COMM: 617/563-9303
FTS: 840-7209

2. Location:

Otis Methods Development Center
Otis ANG base, MA 02542

3. Number and title of CRIS work unit:

WRU #1315-20251-001 Accession # 0043029
"Improve technology for lab culture, mass production and use of the gypsy moth and its natural enemies."

4. Approach Element and Problem Definitions:

2.4.9c.15 - Identify, develop, and improve the use of microbial agents for the control of insect pests through basic research.
2.4.9c.18 - Development of new and improved techniques for the production of microbial agents *in vitro* and *in vivo*.

5. Estimated SY's:

2.4.9c.15 - 0.8 SY
2.4.9c.18 - 0.2 SY

6. Objectives of research: (Broad and long term)

(1) Develop fundamental principles involved in mass production and use of microbial agents (especially Baculoviruses)

(2) Develop a fundamental understanding of environmental, physiological and genetic regulation of virulence and persistence of insect viruses (especially Baculoviruses).

(3) Use knowledge gained from (1) and (2) above to support, improve or develop alternative and environmentally acceptable strategies for insect pest control.

7. Research priorities in your program:

(1) Identify and develop strains of insect pathogens (primarily Baculoviruses) with greater biological activity against insect pest species.

(2) Identify and develop strains of insect pathogens (primarily Baculoviruses) with greater persistence to sunlight inactivation.

(3) Identify and develop new pathogens useful as microbial control agents.

(4) Identify factors regulating transmission of insect pathogens (primarily Baculoviruses) within a given insect generation (= horizontal transmission) and from generation to generation (=vertical transmission).

8. Progress of current research in solving problems:

Several approaches have been utilized to develop a better basic understanding of factors influencing both virus activity and persistence. Moreover, these approaches are leading to a more virulent and environmentally stable virus, which could be more effective as a microbial control agent. Virus activity is influenced by such factors as: site of initial replication, time from infection to harvest, physiological state of the host. By understanding these functions, we may now obtain a more potent virus. Genetic selection has succeeded in obtaining a more UV-stable virus, and will lead also to a more virulent virus.

9. Significant research accomplishments in the past 3 years:

(1) Biological characterization of more than 20 naturally occurring geographical isolates of the gypsy moth NPY (from North America, Europe, and Asia led to the identification of more virulent strains. One isolate, from Abington, MA, is being utilized in basic in vitro studies of virulence, and may be utilized in small-scale field tests.

(2) Two chemicals, e.g., basic acid and sodium borate, were shown to enhance biological activity of the NPY, presumably by acting as stressors.

(3) A UV-tolerant strain of virus was obtained by genetic selection, and is ca 2 x more resistant than the unselected parent.

(4) Various chemicals have been examined as UV-protectants, because of their ability to absorb UV radiation. The chemicals belong to such diverse groups as sun screens, B-vitamins, purines, dyes. A sun screen (benzilidine sulfonic acid), 2B-vitamins (folic acid, riboflaven), and red dye (Congo red) have demonstrated excellent UV protection. The U.S. Forest Service has tested the efficacy of folic acid and will be conducting field tests in 1985. If successful, folic acid will be utilized in the final virus formulation.

(5) Development of a simplified efficient in vivo NPV technology for production of Lymantria NPV.

10. Impact of research accomplishments on science and the general public:

The research has led to the development of a more simplified, efficient *in vivo* virus production system. At present, the rearing and virus-production technology is being utilized in the private sector to produce a product for both forest and home owner usage.

Present research has led to improvements in both the virulence and environmental stability of the gypsy moth NPV. A more active virus isolate, as well as a more active UV-protectant will be field-tested by the U.S. Forest Service. If successful, this research will be instrumental in improving the performance of an insect virus as a microbial control agent. Moreover, the isolate and UV-protectant will then be utilized as the standard by the USFS for control of the gypsy moth. Moreover, this approach is applicable to other entomopathogenic viruses and other pathogens, and may result in decreased usage of conventional pesticides.

11. Obstacles to achieving objectives:

(1) Lack of expertise working in a concerted (team) effort to solve the problem.

(2) Too much of a time lag between successful lab research (ARS) and field testing of promising materials (APHIS, FS).

(3) Lack of fundamental knowledge of factors controlling virulence of insect viruses.

(4) Little basic knowledge on survival and transmission of insect viruses under natural conditions.

(5) Location of research activity is inappropriate for interaction of expertise required to solve the problem.

12. Future lines of needed research and plan for implementation:

(1) Increased emphasis will be placed on mutagenicity, both in *in vivo* and *in vitro* systems. *In vitro* systems will be used to obtain plaque isolates as well as to quantify changes in viral activity.

(2) *In vitro* tissue culture will be employed to determine the effects of host tissue upon viral replication and activity. Data from *in vivo* tests indicates that both quantity and quality of an insect virus is influenced greatly by the insect host (age and stage).

(3) The effect of UV radiation upon viral activity and persistence will be studied by such techniques as electron microscopy and *in vitro* tissue culture; with and without the addition of UV-protectants.

13. Research facilities and personnel needs:

The present location is far from ideal for carrying out research objectives, due to lack of key support personnel, equipment, and lack of other scientists. Because of these inadequacies, much effort has been expended to involve key scientists at other locations. For the long-term, however, it is recommended that this research be carried out at the Beltsville Agricultural Research Center (or other comparable facilities) where expertise in pathology, serology, tissue culture, microscopy, molecular genetics exists.

14. Extent of cooperation--names of persons and institutions:

Dr. Robert Granados - Boyce Thompson Institute
Dr. Edward Dougherty - USDA-ARS, Beltsville, MD
Dr. Franklin Lewis - USDA-FS, Hamden, CT
Dr. Mauro Martignoni - USDA-FS, Corvallis, OR
Dr. William McCarthy - Pennsylvania State University

15. Titles of publications for the last 3 years:

Shapiro, M. 1981. Large scale *in vivo* production of the gypsy moth nucleopolyhedrosis virus at Otis AFB. *In* Doane, C. C. and M. L. McManus (eds.) The Gypsy Moth Research Towards Integrated Pest Management. U.S. Dept. Agric. Tech. Bull. 1584:464-466.

Bell, R. A., C. D. Owens, M. Shapiro, and J. G. R. Tardif. 1981. Development of mass rearing technology. U.S. Dept. Agric. Tech. Bull. 1584:599-633.

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Shapiro, M. , R. A. Bell, and C. D. Owens. 1981. Evaluation of various artificial diets for *in vivo* production of the gypsy moth nucleopolyhedrosis virus. J. Econ. Entomol. 74:110-111.

Shapiro, M., R. A. Bell, and C. D. Owens. 1981. Simplified, efficient system for *in vivo* mass production of gypsy moth nucleopolyhedrosis virus. J. Econ. Entomol. 74:341-343.

Poinar, G. O., Jr., M. Shapiro, and J. E. Lindegren. 1981. Susceptibility of the gypsy moth (*Lymontria dispar*) to the parasitic nematode, *Neoplectora carpocapsae*. IRCS Med. Sci.; Biochem.; Devel. Biol. and Med., Environ. Biol.; Microbiol.; Parasital.; and Infect. Dis. 9:985.

Shapiro, M., and R. A. Bell. 1982. Production of the gypsy moth, Lymantria dispar (L.), nucleopolyhedrosis virus, using carragenons as dietary gelling agents. Ann. Entomol. Soc. Am. 75:43-45.

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Shapiro, M. 1982. In vivo mass production of insect viruses for use as pesticides. In Kurstak (ed.), Microbial and Viral Pesticides. Marcel Dekker, N.Y., pp. 463-492.

Shapiro, M., P. P. Agin, and R. A. Bell. 1983. Ultraviolet protectants of the gypsy moth (Lepidoptera:Lymantriidae) nucleopolyhedrosis virus. Environ. Entomol. 12:982-985.

Shapiro, M., J. L. Robertson, M. G. Injac, R. Katagiri, and R. A. Bell. 1984. Comparative infectivities of gypsy moth (Lepidoptera:Lymantriidae) nucleopolyhedrosis virus isolates from North America, Europe, and Asia. J. Econ. Entomol. 77:153-156.

Shapiro, M. 1984. Host tissues and metabolic products as ultraviolet screens for the gypsy moth (Lepidoptera:Lymantriidae) nucleopolyhedrosis. Environ. Entomol. 13:1131-1134.

Shapiro, M., and R. A. Bell. 1984. Selection of a UV-tolerant strain of the gypsy moth, Lymontria dispar (L.) (Lepidoptera:Lymontriidae), nucleopolyhedrosis virus. Environ. Entomol., in press.

Shapiro, M., G. O. Poinar, J. E. Lindegren. Suitability of Lymontria dispar (Lepidoptera:Lymontriidae) as a host for the entomogenous nematode, Steinermena feltial (Rhabdita:Steinernematidae). Accepted by J. Econ. Entomol.

1. Scientist's name, address, and telephone number:

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2. Location:

Central Plains Area
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Columbia, MO 65205

3. Number and title of CRIS work unit:

CRIS WRU: 3802-20260-024
UTILIZATION OF ENTOMOPATHOGENS FOR POPULATION CONTROL OF PEST INSECTS

4. Approach Element and Problem Definitions:

Technical Objective:	2
Approach:	2.4
Approach Element:	2.4.09.1.b
*Problem:	1
*Subproblem:	b

*No designation on official CRIS (unless the 1.b designator refers to the problem and the subproblem.

5. Estimated SY's:

SY = 1.0

6. Objectives of research:

1. Conduct fundamental studies to explore and evaluate how selected environmental factors affect host-pathogen interactions. Conduct studies to explore and evaluate the introduction, establishment, and fate of entomopathogens in natural and alien ecosystems and the basic mechanisms and strategies involved in the initiation of epizootics. (Tentative SY estimate 60%).

2. To explore and evaluate non-conventional methodologies of insertion of entomopathogenic microorganisms into available agro-ecosystems in accordance with prescribed Agency directives and to determine their real and potential impact on pest species within the agro-ecosystem. (Tentative SY estimate 25%).

3. Conduct fundamental studies on the formulation and application of real and potential entomopathogens selected as candidates for the microbial reduction of pest insect populations and to explore and evaluate their utilization in pest management programs. (Tentative SY estimate 15%).

7. Research priorities in your program:

To develop biorational methods of insect suppression and/or regulation through the use of entomopathogens. Research priorities involve laboratory and field approaches to the utilization of entomopathogens. Host-pathogen associations receive priority; investigations of dose-mortality, modes of infection, pathogenicity, host range, and physical and biological factors affecting these associations are explored. Field studies involving the effects of insect diseases (primarily those caused by the Baculoviruses) on pest populations, required inocula loads, dissemination, and persistence in the agro-ecosystem are of interest. The development of approaches through which available entomopathogens may be used in applied situations through the development of applications techniques is also a primary interest area.

8. Progress of current research in solving problems:

Current investigations are providing information on some of the physical limitations of Baculoviruses and other entomopathogens in row crop ecosystems. Isolation and identification of limiting factors will provide for the maximum utilization of these agents in the regulation of pest insect populations. New pathogens have been isolated and established agents are being explored for use in exotic systems. Exotic baculoviruses are also being considered for use against selected North American insect species.

9. Significant research accomplishments in the past 3 years:

(a) Demonstrated that the incorporation of feeding adjuvants with a viral insecticide (Elcar , Baculovirus heliothis) increased Heliothis zea larval mortality rates 8-10X. Increased activity was determined to be caused by a water soluble component of cottonseed flour; (b) Demonstrated field efficacy of baculovirus (Autographa californica, MEV) against cabbage loopers in MO: (c) Introduced exotic virus (Artogeia (=Pieris) rapae GV) and demonsrated its field efficacy against the imported cabbageworm in MO; (d) Isolated a CPV from the green cloverworm (Plathypena scabra) a potential defoliator of soybeans; (e) Demonstrated compatibility of a fungicide (Kocide) and a fungus (Erynia phytonomi) of alfalfa weevil larvae (Hypera postica) in MO; (f) Conducted extensive formulation and application tests with Elcar (Baculovirus heliothis) and bacterial (Bacillus thuringiensis) in an attempt to improve field efficacy; (g) Successfully advanced the expression of the fungus Erynia phytomoni in small enclosures in an endemic area in addition to recording, delineating, and measuring conidia showers of this fungus; (h) Demonstrated persistence of baculoviruses (2 NPV's and a GV) from soils of endemic areas.

10. Impact of research accomplishments on science and the general public:

The impact of these findings and general investigations is difficult to assess at the current time; however, the results of these research findings have demonstrated the feasibility and potential of entomopathogens (particularly baculoviruses) in pest management schemes along with identifying real and potential limitations in agricultural systems.

11. Obstacles to achieving objectives:

Two significant obstacles are present pursuant to these research objectives: (a) Consistant administrative support in the areas of direction and goals (or missions) resulting in the inability to project comprehensive, long-range planning which will be in cadence with future academic and industrial developments; (b) The difficulty of pursuing these objectives in a sustained, meaningful manner with the continued vagueness of purpose and the very limited and unpredictable fiscal operating restraints.

12. Future lines of needed research and plan for implementation:

Future lines of research will attempt to determine and delineate host-pathogen interactions in natural and alien environments with complimentary epizootical studies designed to enhance the use of entomopathogens in selected management programs. Implementation would follow logical sequences based on the host-pathogen-ecosystem factors.

13. Research facilities and personnel needs:

Although additional facilities and personnel appear high on all lists; current resources may prove to be adequate if facilities and personnel are tailored to accomplish those prioritized lines of research established by the Agency in accordance with current and anticipated National needs.

14. Extent of cooperation—names of persons and institutions:

Current or anticipated cooperative projects are in place with the following individuals and institutions: Dr. A. J. Keaster, Dept. of Entomology, University of Missouri; Dr. R. Brandenburg, Dept. of Entomology, University of Missouri. Recent cooperators include: Dr. M. R. Bell, USDA-ARS-WR, Cotton Res. Lab., Phoenix, Arizona; Dr. N. Dubois, USDA-FS, Forest Insects and Disease Lab., Hamden, Connecticut. Projects range from direct cooperative grants (Dr. Keaster) to collaborative testing (Dr. Dubois).

15. Titles of publications for the last 3 years:

Smith, D.B., and D.L. Hostetter. Laboratory and field evaluation of pathogen-adjuvant treatments. J. Econ. Entomol. 75:472-476. 1982.

Hostetter, D.L., D.B. Smith, R.E. Pinnell, C.M. Ignoffo, and C.H. McKibben. A laboratory evaluation of adjuvants for use with Baculovirus heliothis virus. J. Econ. Entomol. 75:1114-1119. 1982.

Smith, D.B., D.L. Hostetter, F.D. Harris, C.R. Vogt, and R.W. Sommer. Deposition of suspension spray particles. TRANS of the ASAE 25:1141-1147. 1982.

Wilkinson, J.D., K.D. Biever, and D.L. Hostetter. Resistance of cabbage looper Trichoplusia ni (Hubner) (Lepidoptera:Noctuidae) to methomyl in Missouri. J. Kans. Entomol. Soc. January 1983.

Smith, D.B., D.L. Hostetter, and R.E. Pinnell. Laboratory evaluation of plant-derived granules for bollworm control with a virus. (In press) J. Ga. Entomol. Soc. 1983.

Hostetter, D.L.,B. Puttler, J.L. Huggans, R.E. Pinnell, and S.H. Long. Effects of the fungicide Kocide on the entomopathogenic fungus Erynia (=Zoophthora) phytonomi of the alfalfa weevil in Missouri. J. Econ. Entomol. 76:619-621. 1983.

Marston, N.L., D.L. Hostetter, R.E. Pinnell, J.D. Hoffman, and D.B. Smith. Natural mortality of lepidopteran eggs and larvae in Missouri soybeans. Ann. Entomol. Soc. Amer. 77:21-28. 1984.

Hostetter, D.L. and C.M. Ignoffo. Utilization of entomopathogens as controll agents against larvae in various agroecosystems. Chapter10 In "Suppression and Management of Cabbage Looper Populations." USDA Tech. Bul. No. 1684, p. 67-76. 1984.

Ignoffo, C.M. and D.L. Hostetter. Diseases. Chapter 8 In "Suppression and Management of Cabbage Looper Populations." USDA Tech. Bul. No. 1684 p. 45-57. 1984.

Hostetter, D.L. and M.R. Bell. (In Press). Natural dispersal of baculoviruses in the environment. Chapter In "Viral Insecticides for Biological Control" ed. K. Maramorosch and K.E. Shemian. Academic Press, NY, NY.

1. Scientist's name, address, and telephone number:

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2. Location:

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3. Number and title of CRIS work unit:

3802-20260-021: PATHOLOGY AND MICROBIAL CONTROL OF INSECT PESTS

4. Approach Element and Problem Definitions:

2.2.09b: DISCOVER AND EFFECTIVELY USE INSECT PATHOGENS
2.2.05.1b: GENETICS AND GENETIC ENGINEERING OF INSECT PATHOGENS
2.2.01.1h: IN VITRO CULTURE OF INSECT CELLS

5. Estimated SY's:

Current 1.0
Requested 2.5

6. Objectives of research:

RESEARCH MODELS: The Heliothis nuclear polyhedrosis virus (NPV) and pest species of Heliothis.

GENERAL OBJECTIVE:

Develop new and enhance the effective use of microbial insecticides against destructive insects via fundamental and applied research on: the pathogenic microorganism(s); its host(s); the environment; and interactions between these three components.

SPECIFIC OBJECTIVES:

PATHOGEN STUDIES--Increase basic knowledge of the cellular, subcellular, and molecular characteristics of entomopathogens through research on: modification of pathogenic microorganisms by means of genetic manipulation(s); characterization of the infection process(es); characterization of the pathogen's genome; integration of the pathogen's genome into the host genome; extrachromosomal inheritance; basic mechanism(s) of replication; and basic mechanism(s) of environmental inactivation of microbes.

HOST STUDIES--Define and modify endogenous factors that regulate the interactions between host and entomopathogen(s) through fundamental research on: characterization of the host genome; the site and mechanism(s) of resistance and susceptibility to entomopathogens; and the mode of replication of pathogens in homologous and non-homologous hosts.

M. Ignoffo

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IC OBJECTIVES)

characteristics of entomopathogenic

pathogens into the host

ones; basic mechanism(s) of replication

ental inactivation of microbes.

ENVIRONMENTAL STUDIES--Establish how environmental factors affect host-pathogen interactions through research; to evaluate and explore introduction, establishment, and fate of pathogens in natural and alien systems; on basic mechanism(s) and strategies to initiate epizootics; and on innovative strategies and methods for introducing pathogens into agroecosystems.

7. Research priorities in your program:

ESTABLISH IN VIVO AND IN VITRO CELL-LINE MODEL-SYSTEMS FOR STUDY OF THE NUCLEAR POLYHEDROSIS VIRUS OF HELIOTHIS SPECIES.

DETERMINE THE GENETIC MECHANISM AND PHYSIOLOGICAL-PATHOLOGICAL SITE OF RESISTANCE OF HELIOTHIS SUBFLEXA TO THE SINGLE-EMBEDDED NUCLEOPOLYHEDROSIS VIRUS OF HELIOTHIS SPECIES.

DETERMINE THE GENETIC STABILITY OF THE GENOME OF THE HELIOTHIS NUCLEAR POLYHEDROSIS VIRUS BOTH IN VIVO AND IN VITRO.

DETERMINE THE RELATIVE IN VIVO AND IN VITRO SUSCEPTIBILITY OF DOMESTIC AND ALIEN SPECIES OF HELIOTHIS TO THE HELIOTHIS NUCLEAR POLYHEDROSIS VIRUS.

DETERMINE THE SUSCEPTIBILITY OF HYBRIDS OF HELIOTHIS SPECIES TO THE NUCLEOPOLYHEDROSIS VIRUS.

8. Progress of current research in solving problems:

There is systematic progress toward the attainment of current research objectives. The lack of a trained Molecular Biologist-Geneticist to complement our current program is a major impediment to future successful research on the genetic characterization and manipulation of the genome of the nuclear polyhedrosis virus of Heliothis.

9. Significant research accomplishments in the past 3 years:

Established in vivo and in vitro host systems of Heliothis species for the study of gene selection, isolation, and expression of the Heliothis nuclear polyhedrosis virus.

Determined that Heliothis subflexa and its hybrids with H. virescens are resistant to the Heliothis NPV. The first report of high levels of resistance of parents and their hybrids to a baculovirus.

Determined that resistance of H. subflexa to the Heliothis NPV is controlled by a single-gene and that resistance is manifested at mid-gut-epithelial cells.

Characterized the genome, by REN analysis, of the granulosis virus, the multiple-embedded NPV and the single-embedded NPV of Heliothis species. Characterized and defined the genomic stability of three isolates of the single-embedded Heliothis NPV.

Established the in vitro host range of five baculoviruses in homologous and non-homologous cell lines.

10. Impact of research accomplishments on science and the general public:

The successful completion of our research objectives will have both a scientific and societal impact. Scientifically it will provide: novel variants of Baculovirus heliothis that are easier to produce and more effective as microbial insecticides; models, and protocols that can be applied to other promising viral insecticidal candidates; available, alternative strategies to minimize development of resistant populations of Heliothis pests. From a societal perspective it will provide products that: are safer for man; have no deleterious effects on non-target organisms; will not accumulate in or pollute the environment.

There are important reasons why we selected the nuclear polyhedrosis virus, of Heliothis, as the model for our research. This virus attacks species of Heliothis that are major U.S. pests (H. virescens, H. zea) as well as cosmopolitan pests (H. armigera, H. punctigera, H. phloxiphaga). Species of Heliothis attack at least 30 food and fiber crops, require large quantities of broad-spectrum, toxic chemical insecticides for their control (costs of control and losses in the U.S.A. exceed $\$10^9$), and are either resistant to or are developing resistance to the newer synthetic insecticides. Since this virus is registered as a commercial, microbial insecticide any success we achieve in genetically-engineering its genome can be immediately implemented, and any fundamental or applied knowledge attained for the model-system will have a greater impact in solving national and international problems with this pest.

11. Obstacles to achieving objectives:

The Biological Control of Insects Research Laboratory has the staff, model host systems, and facilities to conduct this important research. The only and major obstacle to achieving our objective is lack of funds to support the hiring of a Molecular Biologist-Geneticist. Acquisition of a Molecular Biologist to genetically map and manipulate the nuclear polyhedrosis virus of Heliothis will complement and fortify our current research with this virus. The Biological Control of Insects Research Laboratory has established both in vivo and in vitro model systems of Heliothis for studies of gene transfer and expression and variants of B. heliothis have been characterized both biochemically and by REN analysis.

12. Future lines of needed research and plan for implementation:

OBJECTIVE: Genetically map, characterize and manipulate the genome of Baculovirus heliothis, in an effort to: (a) increase its virulence and rate of mortality against the Heliothis bollworm-budworm complex; (b) broaden its host range to other pests; (c) increase its environmental stability and persistence; and (d) increase its ease and flexibility of production.

IMPLEMENTATION PLAN: The acquisition of fundamental knowledge on the genome is a prerequisite for any attempt to genetically select and engineer Baculovirus heliothis. Novel technologies for genetic manipulation, developed with current industrial and medical microorganisms, are available and directly transferrable to engineering this virus. Thus, it is very probable that Baculovirus heliothis can be made more effective by genetic manipulation.

The initial focus of this research, will be to map, identify and characterize the genome of Baculovirus heliothis i.e., specifically those genes responsible for expressing the desired traits. After completion of the first phase we will:

select, isolate, clone, copy and preserve genes that are controlling traits we desire to transfer; characterize gene structure and expression; and manipulate the desired gene, through selection, recombination and genetic engineering.

In order to successively implement this plan and accomplish our objectives; this research must be done by a team. The proposed Molecular Biologist-Geneticist will be responsible for attaining the objectives detailed above. We are currently staffed with two members of this team i.e., an Insect Virologist and Microbiologist (cf item 13) with collaborative support from a Biochemist. It is imperative that a Molecular Biologist be hired to complement and fortify this team.

13. Research facilities and personnel needs:

Research facilities and basic equipment to conduct research is adequate to attain objectives. What is urgently needed is a Molecular Biologist to complement our currently staffed Insect Virologist (conducting in vivo research) and Microbiologist (conducting in vitro research on specificity, transfection and replication in established lines of Heliothis cells).

14. Extent of cooperation--names of persons and institutions:

Dr. A. H. McIntosh	USDA/ARS/BCIRL, Columbia, MO
Dr. M. D. Huettel	USDA/ARS, Gainesville, FL
Dr. M. E. Martignoni	USDA Forest Service, Corvallis, OR
Dr. R. Rousch	Mississippi State Univ., Mississippi State, MS
Dr. T. L. Couch	Abbott Labs, Inc., Chicago, IL
Dr. J. L. Vaughn	USDA/ARS/Pathology Unit, Beltsville, MD
Dr. C. Quohou	People's Republic of China, Hupeh, China

15. Titles of publications for the last 3 years:

See attached listing.

15. Titles of publications for the last 3 years: (1981-1983 inclusive)

Ignoffo, C. M. 1981. Progress in Microbial Control (1975-1980): Developing Integrated Pest Management Programs for Soybeans" IN Proceedings of Workshop on Insect Pest Management with Microbial Agents: Recent Achievements, Deficiencies, and Innovations, Boyce-Thompson Institute (May 12-15, 1980). 71 pp.

Ignoffo, C. M., C. Garcia, M. J. Kroha, T. Fukuda and T. L. Couch. March 1981. Laboratory Tests to Evaluate the Potential Efficacy of Bacillus thuringiensis var. israelensis for Use against mosquitoes. Mosquito News 41(1) 85-93.

McIntosh, A. H. and C. M. Ignoffo. Replication and Infectivity of the Single-Embedded Nuclear Polyhedrosis Virus, Baculovirus heliothis, in Homologous Cell Lines. J. Invertebrate Pathology 37: 258-264, 1981.

Ignoffo, C. M., T. L. Couch, C. Garcia, and M. J. Kroha. April 1981. Relative Activity of Bacillus thuringiensis var. kurstaki and B. thuringiensis var. israelensis Against Larvae of Aedes aegypti, Culex quinquefasciatus, Trichoplusia ni, Heliothis zea, and Heliothis virescens. J. Econ. Entomol. 74 (2): 218-222.

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Ignoffo, C. M. 1981. [illegible]
live [illegible] [illegible] manage [illegible]
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Plants: Recent Achieve[illegible], Ioffel? [illegible]
a-Thompson Insti[illegible] 12-[illegible]

[illegible]
Viruses. [illegible] Ent [illegible].

Ignoffo, C. M. 1981. [illegible]
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Diseases 1970-1980. Edited by [illegible] D. Bur[illegible]
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Ignoffo C. M. and T. [illegible] Couch. 1981. The [illegible]
of Heliothis Species as a Microbial [illegible]
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H. D. Burges. June/July, 1981. 919 pp. Academic

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Pathogen. Chapter 34. [illegible] Microbial Control of
[illegible]ases. 1970-1980. Edited by H. [illegible]
p. [illegible]-634. Academic Press.

McIntosh Arthur H. and C. M. Ignoffo. 1981. [illegible]
[illegible]ent Baculovirus Infection in a Lepidopteran Ce[illegible]
Invertebrate Pathology 38: 395-403

[illegible]ffo, C. M. 1981. Living Microbial Insecticides
[illegible] in Applied Microbiology, edited by J. R. [illegible]
[illegible]. J. Wiley & Sons [illegible].

[illegible] Arthur H. [illegible] A. [illegible]drews, and Carlo M. Ignoffo. 1981
[illegible] Establishment [illegible] Continuous Cell Lines of
[illegible] (F.) (Lepidoptera: Noctuidae) In Vitro 17:

Bell, James V., Robert J. Hamalle, and Carlo M. Ignoffo. Advances in Agricultural Technology, Southern Series, No. 24, April 1982. Illus., table. "Methods and Costs of Producing Nomuraea rileyi Conidiospores"

Smith, D. B., D. L. Hostetter, R. E. Pinnell, and C. M. Ignoffo. 1982. Laboratory Studies of Viral Adjuvants, Formulation Development. J. Econ. Entomol. 75:16-20.

Ignoffo, C. M., Clemente Garcia, and Michael J. Kroha. 1982. Susceptibility of Larvae of Trichoplusia ni and Anticarsia gemmatalis to Intrahemocoelic Injections of Conidia and Blastospores of Nomuraea rileyi. J. Invertebr. Pathol. 39:198-202.

Ignoffo, Carlo M., Clemente Garcia, Michael Kroha. 1982. NOTES: Susceptibility of the Colorado Potato Beetle Leptinotarsa decemlineata to Bacillus thuringiensis. J. Invertebr. Pathol. 39: 244-246.

McIntosh, A. H. and C. M. Ignoffo. 1982. Notes on a Low-Cost Medium for Growth of Insect Cell Lines and Replication of Insect Viruses. J. KS Entomological Society 55(2):354-356.

Batzer,O. F. and C. M. Ignoffo. June 13, 1978. Pesticide Compositions Stabilized with Sulfonated Catechin/Leucocyanidin Copolymer and Method of Using Same. United States Patent, No. 4,094,969.

Ignoffo, C. M., C. Garcia, M. Kroha, and T. L. Couch. 1982. High-Temperature Sensitivity of Formulations of Bacillus thuringiensis var. israelensis. Environ. Entomol. 11:409-411.

Ignoffo, C. M., C. Garcia, M. Kroha, and T. L. Couch. 1982. Use of Larvae of Trichoplusia ni to Bioassay Conidia of Beauveria bassiana. J. Econ. Entomol. 75: 275-276.

Ignoffo, C. M. 1982. Living Insecticides. p. 125-127. IN Will There Be Enough Food, The 1981 Yearbook of Agriculture, U. S. Dept. of Agriculture, pp. 302.

Ignoffo, C. M. 1982. Keynote Address: "FROM THERE TO HERE TO WHERE" 3rd International Colloquium on Invertebrate Pathology, September 6-10, 1982, University of Sussex, Brighton, United Kingdom. Abstract.

Ignoffo, C. M., Garcia, C., Pinnell, R.E., and Kroha, M. J. 1982. Resistance of Caterpillars to N. rileyi. 3rd International Colloquium on Invertebrate Pathology, September 6-10, 1982, University of Sussex, Brighton, United Kingdom. Abstract.

Ignoffo, C. M. 1982. Environmental Persistence of N. rileyi. 3rd International Colloquium on Invertebrate Pathology, September 6-10, 1982, University of Sussex, Brighton, United Kingdom. Abstract.

Hostetter, D. L., Smith, D.B., Pinnell, R.E., Ignoffo, C. M. and McKibben, G.H. 1982. Laboratory Evaluation of Adjuvants for Use with Baculovirus heliothis Virus. J. Econ. Entomol. 75(6): 1114-1119.

McIntosh, A.H. and C. M. Ignoffo. 1983. Restriction Endonuclease Patterns of Three Baculoviruses Isolated from Species of Heliothis. Journal of Invertebrate Pathology, Vol. 41, pages 27-32.

Ignoffo, C. M., Garcia, C., Kroha, M., and Couch, T. The Effects of Temperature and Water on the Insecticidal Activity and Spore Viability of a Wettable Powder Foundation of Bacillus thuringiensis var. israelensis. J. KS Ent. Soc. 56(1);88-92, 1983.

Ignoffo, C. M., McIntosh, A. H., and Garcia, C. 1983. Susceptibility of Larvae of H. zea, H. virescens, and H. armigera [Lep.: Noctuidae] to 3 Baculoviruses. Entomophaga 28(1): 1-8.

Ignoffo, C. M. 1983. Keynote Address: "FROM THERE TO HERE TO WHERE" 3rd International Colloquium on Invertebrate Pathology, September 6, 1982. University of Sussex, Brighton, United Kingdom. SIP NEWSLETTER Vol. 15(2):11-14.

Ignoffo, C.M., Garcia, C., Kroha, M.J., Samsinakova, A., Kalalova, S. 1983. A Leaf Surface Treatment Bioassay for Determining the Activity of Conidia of Beauveria bassiana against Leptinotarsa decemlineata. J. Invertebr. Pathol., Vol. 41, 385-386

McIntosh, A.H. and C.M. Ignoffo. 1983. Characterization of Five Cell Lines Established from Species of Heliothis. Applied Ent. Zool. 18(2):262-269.

Biever, K. D., G. D. Thomas, P. E. Boldt, and C. M. Ignoffo. Effects of Heliothis zea (Lepidoptera: Noctuidae) on SOybean Yield and Quality. J. Econ. Entomol. 76: 762-765 (1983).

Ignoffo, C. M., R. E. Pinnell, and C. Garcia. Hemocyte Counts in Susceptible and Resistant Noctuid Larvae Injected with Blastospores of N. rileyi. J. KS Ent. Soc. 56(3) 1983, pp. 289-296.

McIntosh, A. H., Carlo M. Ignoffo, Chen Quhou, and Mike Pappas. Establishment of a Cell Line from Heliothis armigera (Hbn.) (Lepidoptera: Noctuidae) In VItro 19(8):589-590, August 1983.

Ignoffo, C. M., Arthur H. McIntosh, Clemente Garcia, Milton D. Huettel, Alfred J. Hill. Relative Resistance of Heliothis subflexa to a Single-Embedded Nucleopolyhedrosis Virus of Heliothis Species. J. Invertebr. Pathol. 42: 282-284, 1983.

Ignoffo, C. M., M. Martignoni, James L. Vaughn (Editors). A Comparison of the US (Gypchek) and USSR (Virin-Ensh) Preparations of the Nuclear Polyhedrosis Virus of the Gypsy Moth, Lymantria dispar. Results of Research Conducted Under Project V-01.0705, Microbiological Control of Insect Pests, of the US/USSR Joint Working Group on the Production of Substances by Microbiological Means. Sponsored by the US/USSR Joint Working Group on the Production of Substances by Microbiological Means and supported by the American Society for Microbiology through a contract with the National Science Foundation. 1983.

United States Department of Agriculture | Agricultural Research Service | National Program Staff | Beltsville, Maryland 20705

June 21, 1984

SUBJECT: Operational Planning on Insect Pathology

TO: Scientists working in Insect Pathology

We are developing documentation as part of the operational phase of strategic planning to try and develop a national program on insect pathology.

Enclosed is a one page survey that is to be provided to all ARS research scientists conducting research or contemplating research utilizing pathogens of insects against insect pest species. If you know of others not on the attached list, please let us know or forward them a copy of this cover letter and survey form. Please prepare a separate sheet for each current or contemplated future research project.

Please forward the completed survey form prior to July 6, 1984 to:

C. M. Ignoffo
USDA-ARS-NPS
Room 237, Bldg. 005
BARC-West
Beltsville, MD 20705

Thanks for your help!

Carlo M. Ignoffo
National Program Staff
Biological Control
(Special Assignment)

Robert D. Jackson
National Program Leader
Entomology

Enclosures

PLEASE TYPE OR PRINT REPLY

INSECT PATHOLOGY OPERATIONAL PLANNING

CURRENT OR FUTURE RESEARCH
(circle one only)

A. Project Title: (Not CRIS title.) Prepare separate sheet for EACH current or future research project.

B. Research Objective: (1-2 sentence statement of what you expect to accomplish)

C. Primary Target Insect Pest Species:

D. Target Pathogen: Genus and Species (List in priority only those you are working with now to achieve the described research objective (above).

1.
2.
3.
4.
5.
6.

E. Intended Primary Use in Commodity or Agroecosystem (e.g. cotton, corn, soybean, pastures, forests, man, animals, etc.)

F. Intended Primary Strategy (circle): Exploration Colonization Augmentation Conservation

G. Problem Type (circle): Laboratory Field Other (describe)

H. Principal ARS Investigator(s): Name(s)

I. CRIS Number and Title:

J. Approach Element/Problem/Subproblem:

K. Number ARS Personnel: SY____; Support-Federal_____; Other_____

L. Current Annual ARS Budget:

M. Estimated Completion Date for this Research Project (circle):

1 year 3 years 6 years greater than 6 years

Insect Pathology

Scientist	Location
B. D. Perkins	Sevres, France
J. R. Adams	Beltsville, MD
T. B. Clark	Beltsville, MD
E. M. Dougherty	Beltsville, MD
R. M. Faust	Beltsville, MD
O. E. Lynn	Beltsville, MD
P. A. W. Martin	Beltsville, MD
W. R. Nickle	Beltsville, MD
R. M. Sayre	Beltsville, MD
G. D. Tompkins	Beltsville, MD
J. L. Vaughn	Beltsville, MD
R. F. Whitcomb	Beltsville, MD
M. Shapiro	Otis AFB, MA
R. A. Humber	Ithaca, NY
R. S. Soper	Ithaca, NY
R. W. Detroy	Peoria, IL
L. C. Lewis	Ankeny, IA
D. E. Johnson	Manhattan, KS
W. H. McGaughey	Manhattan, KS
D. L. Hostetter	Columbia, MO
C. M. Ignoffo	Columbia, MO
A. H. McIntosh	Columbia, MO
M. G. Klein	Wooster, OH
T. L. Ladd, Jr.	Wooster, OH
G. R. Sutter	Brookings, SD
W. E. Burkholder	Madison, WI
D. P. Jouvenaz	Gainesville, FL
L. A. Lacey	Gainesville, FL
A. L. Undeen	Gainesville, FL
C. R. Gentry	Byron, GA
J. A. Payne	Byron, GA
W. L. Tedders	Byron, GA
C. E. Yonce	Byron, GA

James Buchanan

Insect Pathology

Scientist	Location
J. J. Hamm	Tifton, GA
E. I. Hazard	Lake Charles, LA
R. E. McLaughlin	Lake Charles, LA
A. H. Baumhover	Oxford, NC
R. F. Moore	Florence, SC
C. C. Beegle	Brownsville, TX
H. T. Dulmage	Brownsville, TX
D. L. Bull	College Station, TX
R. E. Gingrich	Kerrville, TX
M. R. Bell	Phoenix, AZ
W. R. Kellen	Fresno, CA
J. E. Lindegren	Fresno, CA
P. V. Vail	Fresno, CA
D. E. Meyerdirk	Riverside, CA
D. S. Moreno	Riverside, CA
R. H. Goodwin	Bozeman, MT
J. E. Henry	Bozeman, MT
N. E. Rees	Bozeman, MT

1. Scientist's name, address, and telephone number:

Arthur H. McIntosh
USDA, ARS, BCIRL
P.O. Box A, Research Park, Route K
Columbia, MO 65205

Telephone Number: (314) 875-5361
FTS 276-5361

2. Location:

Central Plains Area
USDA, ARS, BCIRL
Columbia, MO 65205

3. Number and title of CRIS work unit:

CRIS WRU: 3802-20260-022
Genetics, Serology and Biochemistry of Entomopathogens

4. Approach Element and Problem Definitions:

OBJECTIVE: 2
APPROACH: 2.4
APPROACH ELEMENT: 2.4.9; 2.2.01; 2.2.05

5. Estimated SY's:

1

6. Objectives of research:

1. To study the mechanism of host resistance to baculovirus infection at the cellular level employing cell culture methodology.

2. Determine whether or not baculovirus genome integration occurs in infected cell lines.

3. Establishment of new cell lines and hybrids (by cell fusion) for the evaluation of baculovirus replication.

4. To study baculovirus expression in non-permissive cell hosts following challenge with virus.

7. Research priorities in your program:

Research priorities in my program will include:

(1) Studies on the mechanisms of cell resistance to baculovirus infection employing cell cultures.
(2) The in vitro specificity of baculoviruses for lepidopteran cell lines.
(3) The "nature of baculovirus virulence" and how it can be modified to increase its virulence by possible genetic manipulation. This may also include studies into the genetic characteristics of the cell.

8. Progress of current research in solving problems:

Studies on cell resistance to baculovirus infection employing autoradiography and electron microscopy have revealed that the cell membrane may be an important barrier in the infection process. Cell lines from Heliothis species resistant to a baculovirus have been established and are serving as in vitro models to delineate the nature of cell resistance.

Specificity studies on baculoviruses have revealed that the multiple enveloped virus in some cases do not have a wider in vitro host spectrum than the single enveloped viruses which have been thought to be more specific.

9. Significant research accomplishments in the past 3 years:

Significant accomplishments over the past three years include: Establishment of a cell line from a species of Heliothis that is resistant to a baculovirus. This will provide an in vitro model for investigating the nature of virus resistance at the cellular level. The demonstration that with continued passaging of H. zea baculovirus there is no decrease in virulence after 20 in vivo passages and that the genomic pattern, as measured by restriction endonuclease analysis, remains basically the same. The stability of viruses used in biological control is an important factor in their successful usage. The application of the isoelectric focusing technique for the proper identification of insect cell lines. This technique has reduced the time and labor over other methodologies used in the identification of insect cell lines.

10. Impact of research accomplishments on science and the general public:

Impact of this research on the general public is probably negligible because of the basic nature of the work. The impact on the scientific community can be assessed by the many requests for "new" insect cell lines established by this investigator; requests for tissue culture and virology methodologies and requests by scientists to work in this laboratory to acquire "hands on" experience.

11. Obstacles to achieving objectives:

Technical assistance which is limited by fiscal resources.

12. Future lines of needed research and plan for implementation:

Future lines of needed research should include:

(1) Basic mechanism of cell resistance to virus infection employing cell culture methodology.
(2) The in vitro specificity of baculoviruses for lepidopteran cells grown in culture.
(3) The establishment of hybrid cells and their evaluations as competent hosts for the replication of baculoviruses.
(4) Genetic manipulation of virus a/o host to increase virulence of virus or host susceptibility.

Implementation of these research areas will be greatly dependent on available qualified technical assistance.

13. Research facilities and personnel needs:

Additional laboratory space to facilitate working conditions. Acquisition of a graduate student and a post-doctoral fellow would greatly improve research capability.

14. Extent of cooperation — names of persons and institutions:

Dr. Shanti L. Bilimoria
Department of Biological Sciences
Texas Tech University
Lubbock, Texas 79409

Dr. Fred W. Hink
Department of Entomology
Ohio State University
1735 Neil Avenue
Columbus, Ohio 43210

Dr. Carlo M. Ignoffo
USDA, ARS, BCIRL
P.O. Box A, Research Park, Route K
Columbia, MO 65205

15. Titles of publications for the last 3 years:

McIntosh, A. H., and C. M. Ignoffo. Replication and infectivity of the single-embedded nuclear polyhedrosis virus, Baculovirus heliothis in homologous cell lines. J. Invertebr. Pathol. 37:258-263, 1981.

McIntosh, A. H., and C. M. Ignoffo. Establishment of a persistent baculovirus infection in a lepidopteran cell line. J. Invertebr. Pathol. 38:395-403, 1981.

McIntosh, A. H., P. A. Andrews, and C.M. Ignoffo. Establishment of two continuous cell lines of Heliothis virescens (F) (Lepidoptera: Noctuidae). In Vitro:17(18):649-650, 1981.

McIntosh, A. H., and C. M. Ignoffo. A medium for culturing insect cell lines and replicating insect viruses. J. Kans. Entomol. Soc. 55:354-356, 1982.

Ignoffo, C. M., A. H. McIntosh, C. Garcia, M. Kroha, and J. M. Johnson. Effects of successive in vitro and in vivo passages on the virulence of the entomopathogenic fungus, Nomuraea rileyi. Entomophaga 27(4):371-378, 1982.

McIntosh, A. H., and C. M. Ignoffo. Restriction endonuclease patterns of three baculoviruses isolated from species of Heliothis. J. Invertebr. Pathol. 41:27-32, 1983.

McIntosh, A. H., and C. M. Ignoffo. Characterization of five cell lines established from species of Heliothis. Appl. Ent. Zool. 18:262-269, 1983.

McIntosh, A. H., C. M. Ignoffo, C. Quhou, and M. Pappas. Establishment of a cell line from Heliothis armigera (Hbn.) (Lepidoptera: Noctuidae). In Vitro 19(8):589-590, 1983.

Ignoffo, C. M., A. H. McIntosh, C. Garcia, M. D. Huettel, and A. J. Hill. Relative resistance of Heliothis subflexa to a single-embedded nucleopolyhedrosis virus of Heliothis species. J. Invertebr. Pathol 42:282-284, 1983.

Ignoffo, C. M., A. H. McIntosh, and C. Garcia. Susceptibility of larvae of Heliothis zea, H. virescens, and H. armigera (Lep.: Noctuidae) to three baculoviruses. Entomophaga 28(1):1-8, 1983.

Questionnaire: Present Technology in Insect Virus Research in ARS

1. Scientist's name, address, and telephone number:

Ronald H. Goodwin
USDA/ARS
Rangeland Insect Laboratory
Montana State University
Bozeman, Montana 59717-0001
Telephone: (406) 994-3051

2. Location:

USDA/ARS
Rangeland Insect Laboratory
Montana State University
Bozeman, Montana 59717-0001

3. Name and title of CRIS work unit:

5720-20240-004 - Biological Control of Great Plains Grasshoppers

4. Approach element and problem definitions:

Reduce losses - Weeds, disease, insects, nematodes, and insects/disease/nematode losses - Range, pasture, forage, turf

Subproblem: Current technologies are inadequate to develop effective and safe means of protecting rangelands against losses caused by destructive insects and plant diseases.

5. Estimated SY's:

0.2

6. Objectives of research:

Rationalization and improvement of the biological control of pest species of grasshoppers and crickets.

7. Research priorities in your program:

A. Definition of the general requirements of normal cells from Acridid orthopterans for their culture in vitro.

B. Survey of cultured cells from various grasshopper life stages to define which tissue or cell types are able to support entomopoxvirus replication in vitro.

C. Definition of the special requirements for the growth of viral replicating tissue types in culture and the specific requirements of the entomopoxvirus infected cell which will permit the completion of the viral replication cycle.

D. Broadening of tissue culture program to include at least one host species for each present Acridid entomopoxvirus isolate.

E. Initiate in vitro virus strain isolation and characterization followed by in vitro and in vivo host range correlations and extensions.

8. Progress of current research in solving problems:

Study of various tissue culture systems has revealed unique differences between the requirements of cells from the Acrididae and the cultured cells from various other orders of insects. Long-term maintenance cultures of various acridid cell types are presently at the limits of the technology. The development of useful cell lines from these insects for research or industrial purposes will require sigificant advances in insect tissue culture systems, probably requiring new inputs from insect physiology and neuroendocrinology as well as an application of the newest discoveries in vertebrate tissue culture science. These advanced technological inputs are presently under investigation at this laboratory.

9. Significant research accomplishments in the past 3 years:

A. Tissue culture systems advancements in this laboratory have resulted in the first replication of an entomopoxvirus and a unicapsid baculovirus in insect cells grown in a serum-free tissue culture medium.

B. Cell lines from various orders including species related to the grasshoppers (cockroach cell lines) have been cultured for the first time in serum-free (semi-defined) culture systems.

C. The successful new culture systems developed in (B.) have been used to develop low-level serum containing maintenance cultures of grasshopper hemocytes, dissociated embryos, and combined ovary and connective tissues.

10. Impact of research accomplishments on science and the general public:

The development of safe insect microbial control agents, including the insect viruses, will advance pest control science and give us additional tools for the management of integrated control systems which minimize or eliminate toxic residues and pest species genetic resistance problems.

11. Obstacles to achieving objectives:

Time required to develop the necessary cell lines which are capable of replicating the entomopoxviruses from grasshoppers.

12. Future lines of needed research and plan for implementations:

A. Movement of insect virus production from the area of basic toward applied research and commercial development.

Plan: Continuation of research cooperative agreement with NSF grantee investigating the large-scale culture of insect viruses in commercial pharmaceutical and biochemical products level fermentation systems.

D. Broadening of plant [illegible] species for each [illegible]

E. Initiate [illegible] by [illegible]

Progress [illegible]

Study of various [illegible] between [illegible] from [illegible] various [illegible] The development of [illegible] industrial [illegible] culture [illegible] vertebrate [illegible] present [illegible]

[illegible]

A. [illegible]

B. [illegible]

C. [illegible] tissues.

[illegible]

The development of [illegible] insect vectors [illegible] tools [illegible] eliminate [illegible]

Obstacles to achieving objectives

Time required to develop [illegible] application [illegible]

Future lines of needed research [illegible]

A. Movement of insect [illegible] application [illegible]

Plan: (Continuation of [illegible]) investigating the [illegible] phytochemical and biochemical [illegible] systems.

B. Definition of specific biochemical factors influencing the survival and growth of grasshopper cells in vitro.

Plan: Initiation of cooperative agreement with MSU biochemist grantee studying the hemagglutinins produced in acridid grasshoppers (using tissue culture systems developed in this laboratory).

13. Research facilities and personnel needs: (Presently adequate as detailed here)

A. Tissue culture laboratory supported by insectary for rearing grasshoppers.

B. One full-time technician plus student aid time totalling an additional 30-40 hours weekly.

14. Extent of cooperation--names of persons and institutions:

A. Mr. Stefan A. Weiss
Process Development
Cetus Corporation
1400 Fifty-Third Street
Emeryville, CA 94608

B. Dr. W. Fred Hink
Department of Entomology
The Ohio State University
1735 Neill Avenue
Columbus, OH 43210
(NSF grantee receiving grant transferred from Mr. Stefan A. Weiss, above)

C. Dr. Kenneth D. Hapner (biochemist)
Chemistry Department
616 Johnson Hall
Montana State University
Bozeman, MT 59717-0001

15. Titles of publications for the last 3 years:

A. 1982. Potential use of the saltmarsh caterpillar as a production host for nuclear polyhedrosis viruses.

B. 1982. Baculovirus replicating insect cell lines from the bollworm, Heliothis zea: characterization and virus studies.

C. 1982. Characterization and culture of virus replicating continuous insect cell lines from the bollworm, Heliothis zea (Boddie).

D. 1983. Growth of the lepidopteran cells in serum-free medium.

E. 1983. Comparative culture of insect cells from the Lepidoptera, Coleoptera, and Orthoptera in various media.

F. 1984. Recognition and diagnosis of diseases in insectaries and the effects of disease agents on insect biology.

G. 1984. Replication of Heliothis zea baculovirus in insect cells grown in serum-free media (SFM).

H. 1984. Entomopoxvirus and baculovirus replication in serum containing and serum-free cultures of insect cell lines.

Statement: "How genetic manipulation of viruses could enhance their effectiveness in insect control."

Previous experimentation on the enhancement of the virulence of insect viruses was inconclusive, since the (baculovirus) improved strains could not be followed in field studies. Experimentation on the broadening of the host range of insect viruses has also been inconclusive since laboratory studies showed only a shift away from original hosts toward improved infection rates in other hosts, but not an actual broadening of host range in any one virus isolate. We may conclude from the published evidence that serial host passage is probably superior to genetic manipulation in maintaining or improving virulence in most pathogens of humans or of insects. Commercial virus products containing more than one virus strain or species will at present obtain economic viral suppression of susceptible related insect pest species more reliably than genetically engineered virus products. Considerably more sophisticated research will be necessary to define the viral genetic elements responsible for (a) virulence and (b) host range. When this is accomplished then we will be able to ask the question posed above, i.e. whether genetic manipulation has any merits recommending it for the "enhancement" of insect virus control effectiveness over and above serial host passage techniques.

1. Scientist's name, address, and telephone number:

J. E. Henry
USDA/ARS/Rangeland Insect Laboratory
Montana State University
Bozeman, Montana 59717-0001
Telephone: (406) 994-6401 or FTS: 585-4220

2. Location:

Bozeman, Montana 59717-0001

3. Number and title of CRIS work unit:

5720-20240-004 - Biological Control of Great Plains Grasshoppers

4. Approach Element and Problem Definitions:

- Reduce Losses - Weeds, disease, insects, nematodes, and insects/disease/nematode losses - Range, pasture, forage, turf

- Subproblem: Current technologies are inadequate to develop effective and safe means of protecting rangelands against losses caused by destructive insects and plant diseases.

5. Estimated SY's:

Henry - 0.4 (on viruses)

6. Objectives of research: (Entomopoxviruses only)

1. To characterize the entomopoxviruses isolated from grasshoppers.
2. To enhance the virulence and/or host range of particular entomopoxviruses through selection and/or genetic manipulation.
3. To evaluate the applied potential of entomopoxviruses against grasshoppers.

7. Research priorities in your program: Entomopoxviruses are the major virus type in grasshoppers. Currently we have about 8 isolates. Other isolates are known from Argentina and mainland China grasshoppers. The short-term priorities are to characterize the viruses and to develop immuno-diagnostic or labelled DNA probes for rapid and sensitive differentiation of the isolates. The long-term objective is to enhance the virulence and/or host-ranges of selected isolates through selection or genetic manipulation.

8. Progress of current research in solving problems: Research by the pathology unit of this laboratory currently is being redirected from field oriented field tests with Nosema locustae to laboratory studies of entomopox viruses and other protozoa. Recent studies using restriction enzymes have established that the Oedaleus entomopoxvirus (OPV) from Africa is different from several entomopoxviruses isolated from U.S. grasshoppers.

9. Significant research accomplishments in the past 3 years: Partial characterization of 3 isolates from U.S. grasshoppers with restriction enzymes confirmed the indication of specificity from cross-infectivity studies and inclusion body protein electrophoretic profiles. Field studies have established that the Melanoplus entomopoxvirus (MPV) can be applied on a wheat bran carrier and will function in control of certain species of grasshoppers.

10. Impact of research accomplishments on science and the general public: This is the only insect research unit in the world with such a large number of closely related viruses in one family of insects in which there are instances of cross-infectivity. This provides a unique opportunity for developing and comparing the genomes of such viruses and evaluating the effects of genetic interchanges. In a general sense, we have the opportunity to "develop" a viral microbial that might be useful as a short-term control tactic as opposed to the present status of these organisms as long-term management tactics.

11. Obstacles to achieving objectives: The major obstacle is lack of an in vitro (cell culture) growth system. This is important for studies of viral replication, but is particularly important to viral cloning and maintenance of the cloned viral types. Cloning can be accomplished in vivo with considerably more difficulty and in vivo maintenance runs the risk of contamination of the cloned virus with another "accidentally" infecting virus.

12. Future lines of needed research and plan for implementation: These will be determined following completion of some of these initial research efforts to evaluate the potential for meaningful genetic manulations. Also, it will depend to some extent on the successful development of a usable cell culture.

[illegible] research [illegible] in the world with [illegible]
[illegible] closely related viruses in one family of [illegible]
[illegible] mechan[illegible] [illegible]-interchangeability. This provides [illegible]
[illegible] and comparing the genomes of such viruses [illegible]
[illegible] interchanges. In a general sense, we have [illegible]
[illegible] a viral microbial that might [illegible]
[illegible]-term control tactic as opposed to the present status of those
as long-term management tactics.

Challenging [illegible] The major obstacle to [illegible]
virus (cell) cultures [illegible]. This is [illegible]
viral replication, but is necessarily [illegible]
maintenance of the clonal viral types. Clonal [illegible]
vivo will considerably more difficulty and [illegible]
risk of contamination of the cloned virus with [illegible]
infecting virus.

efforts to evaluate [illegible] potential for meaningful genetic manipulation.
Also, it will depend to some extent on the successful development of a
usable cell culture.

13. **Research facilities and personnel needs:**

Present facilities and personnel are adequate for these initial phases. Personnel include J. E. Henry (GM-14), E. A. Oma (GS-11 Cat. 3), Leslie Rykels (GS-5 grasshopper rearing), Dr. D. A. Streett - (M.S.U. Research Associate), and Ann Holland - (M.S.U. lab assistant).

14. **Extent of cooperation--names of persons and institutions:**

Dr. Lois Miller, University of Idaho, currently is providing training to Dr. D. A. Streett for DNA research on entomopoxviruses. (She works on NPV from Autographa.)

15. **Titles of publications for the last 3 years:** (viruses only

Langridge, W. H. R. and Henry, J. E. Molecular weight and base composition of DNA isolated from Melanoplus sanguinipes entomopoxviruses. J. Invertebr. Pathol. 37:34-37. 1981.

Langridge, W. H. R., Oma, E. A., and Henry, J. E. Characterization of the DNA and structural proteins of entomopoxviruses from Melanoplus sanguinipes, Arphia conspersa, and Phoetaliotes nebrascensis (Orthoptera). J. Invertebr. Pathol. 42:327-333. 1983.